THE COMPARATIVE NUTRITIVE VALUES OF GLUCOSE, FRUCTOSE, SUCROSE AND LACTOSE WHEN INCORPORATED IN A COMPLETE DIET

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There are many indications in the literature that the various sugars, ingested separately and without admixture with other nutrients, are absorbed at different rates, are utilized in different ways quantitatively, and exert different physiological effects. They differ in the rate with which they are oxidized or are converted into glycogen or into fat, or in the extent to which they are utilized in various ways. Their specific dynamic effects, either the total effect, the peak effect, or the time curve, have been observed to be somewhat different, and the sugars seem to vary also in ketolytic and protein-sparing capacity. While the experimental evidence on these various points is not always concordant, the fact that significant differences exist is clear.

In its favorable effect upon the absorption and utilization of calcium and phosphorus, lactose stands apart from all other sugars, as the work of Bergeim ('26), Kline, Keenan, Elvehjem and Hart ('32), and of Robinson, Huffman and Mason ('29) in particular prove. This favorable effect seems to be a result of the tendency of lactose to maintain a higher hydrogen ion concentration throughout the intestinal tract by favoring the propagation of an aciduric flora, though other factors also seem to operate in this direction.

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But the fact that sugars when fed alone exhibit individual effects does not imply that when fed as components of a well-balanced diet they will still react differently on the body or be utilized in different ways or to different extents within it, except as these individual effects relate to conditions obtaining in the intestinal tract. What the cells and tissues of the body do to a sudden influx of a particular sugar from the intestinal tract may not determine what they will do to the same sugar accompanied by all other nutrients needed for the maintenance of life, the promotion of growth and the performance of secretory functions. In the former case, in which sugars are fed alone, the disposal of the sugar may follow the path of least resistance, determined by the constitution of the particular sugar fed. In the latter case, with the possibility of manifold anabolic reactions, the same sugar may be used in a more purposeful manner, since its energy and its substance may now be utilized in the promotion of syntheses and interconversions of other nutrients. It is not inconceivable that the various sugars, within certain ranges of dietary concentration at least, are of identical value when incorporated in a diet thus providing all of the nutrients essential for all prevailing body functions. The expectation that the effects of nutrients determined by the feeding of each separately, are necessarily summated when they are combined in a balanced diet, is fundamentally unsound and demonstrably erroneous in many instances, for example, their specific dynamic effects and net energy values.

A number of investigations have been concerned with the nutritive evaluation of balanced diets differing only in the kind of carbohydrate contained in them. Unfortunately for the purposes of this discussion, most of these experiments involved no control of the amounts of food consumed by comparative animals and, for unascertained reasons, the rate of food consumption has quite often differed markedly among different experimental groups. The interpretation of the differences in the rates of gain (or in the composition of the gain) observed, in terms of differences in the inherent nutritive value of the sugars, is difficult if not impossible. Even

when the differences in food intake among comparative animals with unrestricted access to food happen to be apparently slight, the observed differences in experimental measurements may, partly as a result of the failure to control food intake, be so variable as to obscure possible small differences in the nutritive value of the sugars tested. Thus, in the experiments of Speirs and Sherman ('36), it was found that the amounts of calcium and phosphorus retained in the bodies of rats were not statistically different whether they were grown upon diets containing one-third glucose, or dextrin, or cornstarch, or sucrose, or a spray-dried corn sirup containing a mixture of glucose, maltose and dextrin. However, the variations observed in the mineral retentions of the various groups of experimental rats were such that it is impossible to distinguish statistically between the calcium or phosphorus balances of the male rats on the basal diet alone, and those of the male rats on the basal diet diluted with one-half its weight of the various carbohydrates tested. The latter diets contained one-third less calcium and phosphorus than the undiluted basal diet. With the female rats significant differences were observed in only seven of the ten comparisons between the basal diet and the sugar diets.

The experiment of Feyder ('35) comparing the nutritive values of sucrose and glucose was planned according to the paired-feeding method. The sugars constituted 68% of the experimental diets, which were adequately provided with protein, minerals and the vitamins contained in cod liver oil and yeast. The rations were carefully equalized with respect to energy, and at the termination of the experiment, after 16 to 29 weeks of feeding, seven pairs of the surviving rats were killed and their composition compared with that of control rats sacrificed at the beginning of the experiment. Under the conditions of this well-controlled experiment, the sucrose diet promoted more rapid gains in weight than the glucose diet, principally because of a more rapid deposition of fat, but also because of a significantly greater deposition of protein. The formation of glycogen was not significantly different between

the two groups of rats. Unfortunately the experimental rations were not completely balanced, apparently, according to the author, because of a deficiency in unsaturated fatty acids. Most of the rats, sometime during the feeding period, showed tail and skin symptoms similar to those described by Burr and Burr ('29) as evidence of a fat-deficiency disease.

In controlled feeding tests with rats and pigs, Whittier, Cary and Ellis ('35) compared the nutritive values of lactose and sucrose, using rations containing 30% (rats) or 40% (pigs) of sugar. In the rat experiments, in which ten pairs of animals were used, the rate of growth on the two experimental rations was practically identical, as was also the chemical composition of the carcasses as far as may be judged from the analyses of only two pairs. In the pig experiments, twelve animals were fed in four groups of three each; one pig in each group received the lactose ration, one the sucrose ration and one a 'control' ration the carbohydrate of which was derived entirely from brewer's rice. The pigs were fed individually and those in each triplet were fed the same amount of ration, determined by the pig consuming the least. During a feeding period of 25 weeks, the average daily gains in body weight averaged 0.68 pounds for the control pigs, 0.54 pounds for the sucrose pigs and 0.52 pounds for the lactose pigs, but the differences in rate of gain among the different groups are not statistically significant. Also the chemical analyses of carcasses revealed a higher fat content and a lower protein content of the pigs on the sucrose ration as compared with those on the lactose ration, though only three of the sucrose pigs were analyzed. The slow gains of the pigs suggest that the rations may not have been properly balanced.

Neither Feyder nor Whittier, Cary and Ellis consider the possibility of a difference in the digestibility of rations containing different carbohydrate components.

The experiments reported in this paper are concerned with a comparison of the nutritive value of glucose and sucrose, glucose and fructose, and glucose and lactose.

DESCRIPTION OF THE EXPERIMENTS

The experiments were performed upon growing rats, which were fed in accordance with the paired-feeding technic. During the feeding period, one or more determinations of the digestibility of the experimental rations were made, and in one comparison (glucose versus sucrose) qualitative tests were made for the presence of sugar in the urine. In the comparison of glucose and fructose, the paired rats were put in revolving cages, equipped with revolution counters, for periods of 6 to 8 weeks for the purpose of detecting any differential effect of the experimental rations on the voluntary activity of the animals. At the termination of the feeding periods all surviving rats were sacrificed, the contents of the gastro-intestinal tract removed, the empty carcasses weighed, frozen solid and ground finely while still frozen. The samples thus prepared were analyzed for moisture, fat (ether extract), protein ($N \times 6.25$), ash, calcium and phosphorus (except for the rats in the comparison of glucose and sucrose). Also, the heat of combustion (gross energy) of all samples was determined in a Parr oxygen-bomb calorimeter. All gains in body weight were computed from the initial live weight, representing the average of weights taken on three consecutive days, and the final empty weight.

The experimental rations were planned to contain from 60 to 70% of the various sugars, to be adequately balanced, and to be equal in protein and energy. Their composition is given in table 1.

The sugars used in these rations were analytical reagent or C.P. grade, except that the fructose was of only 90% purity. They possessed the following heats of combustion expressed in calories per gram: glucose, 3.699, sucrose, 3.955, fructose, 3.704, and lactose, 3.702. At the time the experiment was undertaken, it was not suspected that the sugars would be likely to contain appreciable amounts of calcium or phosphorus, or that the experimental diets would be likely to exert a differential effect on the retention of these elements in the body. Since such differential effects did in fact appear, the

question arose as to whether they could have been the result of the presence of these minerals in the sugars in variable concentrations. The same or similar supplies of the sugars were therefore analyzed for calcium and phosphorus. No detectable amounts were present in the sucrose, and extremely minute amounts in the glucose (0.000008% phosphorus and 0.00042% calcium). The lactose contained 0.0010% phosphorus and 0.019% calcium, while the fructose contained

TABLE 1

Composition of rations

CONSTITUENTS	RATION 1 (SUCROSE)	RATION 2 (GLUCOSE)	RATION 3 (FRUCTOSE)	RATION 4 (GLUCOSE)	RATION 5 (LACTOSE)
Casein	18	18	..	..	..
Dried whole egg	..	..	25	25	25
Salt mixture	4	4	4.5	4.5	4.5
Cod liver oil	2	2	2	2	2
Yeast	6	6	6	6	6
Glucose	..	70	..	60	..
Sucrose	66.4	..	..	..	..
Fructose	..	..	60	..	..
Lactose	..	..	..	..	60
Agar	..	..	2.5	2.5	2.5
Water	3.6	..	..	..	..
Average calories per gram	3.956	3.969	3.990	3.978	4.018

0.00027% phosphorus and 0.056% calcium. The latter contamination is equivalent to an intake of 3.4 mg. of calcium per 10 gm. of ration.

The experimental rations contained about 0.68% calcium and 0.71% phosphorus.

THE RESULTS OF THE EXPERIMENTS

Glucose versus sucrose. The nutritive values of glucose and sucrose were compared with nine pairs of rats. The essential results of the feeding experiments and the carcass analyses are collected in table 2. In this comparison, ration 1 was compared with ration 2.

TABLE 2

The results of the comparison of glucose and sucrose

RAT NO. AND SEX	SUGAR TESTED	DAYS ON TEST	INITIAL BODY WEIGHT	FINAL EMPTY WEIGHT	GAIN IN WEIGHT	TOTAL FOOD CONSUMED	COMPOSITION OF CARCASS					
							Moisture	Protein	Fat	Energy content	Ash	Calcium
			gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*calories*	*gm.*	*gm.*
85 ♀	Sucrose	76	74	192	118	740	112.7	35.3	37.2	535	7.10	2.03
86 ♀	Glucose	76	70	202	132	740	122.5	39.5	34.5	520	7.35	2.06
87 ♂	Sucrose	83	73	271	198	1035	156.8	56.7	50.5	753	9.34	2.52
88 ♂	Glucose	83	68	277	209	1035	167.7	56.0	44.6	699	8.80	2.36
89 ♀	Sucrose	92	60	169	109	788	105.9	35.6	20.8	380	7.54	2.20
90 ♀	Glucose	92	55	174	119	788	101.5	33.2	33.8	483	6.33	1.72
91 ♂	Sucrose	69	69	263	194	835	156.8	51.7	47.5	706	8.77	2.41
92 ♂	Glucose	69	70	265	195	835	156.2	50.1	50.9	739	8.45	2.28
93 ♀	Sucrose	75	55	192	137	785	113.2	36.9	35.1	511	7.32	2.09
94 ♀	Glucose	75	58	191	133	785	119.7	38.6	26.4	442	7.06	2.04
95 ♀	Sucrose	69	64	193	129	718	116.8	37.6	32.2	492	7.47	2.18
96 ♀	Glucose	69	63	195	132	718	121.0	37.7	29.3	475	7.11	1.96
97 ♂	Sucrose	69	50	222	172	722	143.6	46.0	25.9	479	7.39	2.11
98 ♂	Glucose	69	52	214	162	722	124.7	40.3	45.3	614	6.52	1.75
99 ♀	Sucrose	69	48	170	122	669	102.5	30.8	29.4	448	6.24	1.90
100 ♀	Glucose	69	53	175	122	669	101.2	31.8	37.1	507	5.61	1.58
101 ♀	Sucrose	69	40	178	138	684	106.5	33.9	29.1	457	6.98	2.05
102 ♀	Glucose	69	41	167	126	684	100.9	32.4	26.9	436	6.13	1.70
Averages	Sucrose		59.2	205.6	146.3	775.1	123.87	40.50	34.18	529.0	7.572	2.166
	Glucose		58.9	206.7	147.8	775.1	123.93	39.94	36.53	546.1	7.040	1.939

On equal amounts of food, the gains in body weight were practically identical on the two rations. The contents of the carcasses of pair mates in moisture, protein and fat are not distinguishable statistically, and averaged very nearly the same for the rats on glucose and on sucrose rations. The gross energy contents of the carcasses of pair mates evidently were not affected by the difference in the carbohydrate components of the rations compared. However, with respect to ash and calcium deposited in the bodies of the experimental rats, the storage was greater in eight of the nine pairs for the rat on the sucrose diet. A statistical analysis of the paired differences according to the method of Student ('08) shows clearly that the differences must have been the result of the

TABLE 3

Average percentage composition of the glucose and sucrose rats

RATION	MOISTURE	PROTEIN	FAT	ASH	CALCIUM	ENERGY IN CALORIES PER GRAM
Sucrose	60.30	19.61	16.51	3.72	1.071	2.561
Glucose	59.92	19.28	17.67	3.43	0.947	2.642

difference in experimental treatment imposed. For the ash content, the mean difference between pair mates was 0.532 gm. in favor of the sucrose rat, the standard deviation of differences was 0.401 gm., and the probability of a fortuitous outcome only 0.0029. For the calcium contents, the mean difference was 0.227 gm., the standard deviation 0.155 gm., and the probability 0.0016. Both probabilities are so small that they may be neglected. The differences not being fortuitous, must have been caused by the difference in the composition of the diets, since the amounts consumed by pair mates were identical. In these tests, therefore, sucrose induced a more complete retention of ash and of calcium from diets presumably containing generous amounts of minerals, than did glucose.

The percentage composition of the empty carcasses showed essentially the same differences as the absolute composition. The averages only are given in table 3. Again, only the ash

($m = 0.292$, $s = 0.216$, $P = 0.024$) and the calcium ($M = 0.124$, $s = 0.086$, $P = 0.0030$) differences are statistically significant.

The urines of the experimental rats were tested qualitatively for reducing sugars, using Benedict's solution. When the collections were taken before feeding, no reducing sugars were indicated, but when taken for a few hours after feeding, the urines from the sucrose rats invariably contained reducing sugars while those from the glucose rats were still sugar free.

Digestion trials clearly indicated that the fecal waste from the sucrose rats was significantly greater than that from the glucose rats, consuming the same amount of food, with respect to dry matter, nitrogen and energy. In a final test made upon all nine pairs of rats the fecal dry matter averaged 14.2% greater for the sucrose rats, the fecal nitrogen 25.5% greater, and the fecal energy 17.9% greater. The differences were in the same direction in all pairs. However, the digestibility of the food was evidently good in all rats, since the coefficients for energy averaged 96.20 for the sucrose ration and 96.80 for the glucose ration. The differences in the digestibility of the rations were slight, though highly significant statistically.

Glucose versus fructose. The results of the comparison of glucose and fructose (rations 4 and 3) are summarized in table 4. On the same amounts of food glucose rats gained in weight faster than the fructose rats in seven of the eight pairs. The mean difference in total gain between pair mates was 8.63 gm., the standard deviation of differences was 6.24 gm., and the probability of a fortuitous result was only 0.0041. Of the nutrients in the empty carcasses, the water content was greater in all pairs for the glucose rat ($M = 8.55$, $s = 3.47$, $P < 0.0002$), and the protein content was greater for the glucose rat in seven of the eight pairs ($M = 2.76$, $s = 1.48$, $P = 0.0009$). In respect to fat and energy, the fructose rats contained on the average somewhat more than the glucose rats, but the differences were quite insignificant statistically, since they displayed no consistency in direction among the various

TABLE 4

The results of the comparison of glucose and fructose

RAT NO. AND SEX	SUGAR TESTED	DAYS ON TEST	INITIAL BODY WEIGHT	FINAL EMPTY WEIGHT	GAIN IN WEIGHT	TOTAL FOOD CONSUMED	COMPOSITION OF CARCASS						
							Moisture	Protein	Fat	Energy content	Ash	Calcium	Phosphorus
			gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*calories*	*gm.*	*gm.*	*gm.*
143 ♂	Fructose	50	55	246	191	701	154.1	47.5	37.2	579	8.00	2.24	1.53
144 ♂	Glucose	50	53	258	205	701	159.6	51.1	39.5	641	7.51	2.02	1.42
145 ♀	Fructose	70	51	186	135	746	108.0	35.7	35.1	513	6.72	2.11	1.32
146 ♀	Glucose	70	53	195	142	746	116.7	37.3	34.6	517	7.17	2.11	1.36
147 ♂	Fructose	63	44	225	181	784	143.2	45.5	27.9	500	8.43	2.50	1.61
148 ♂	Glucose	63	45	221	176	784	145.1	45.2	23.9	448	7.98	2.25	1.50
149 ♀	Fructose	78	40	168	128	849	103.7	32.0	26.4	407	6.85	2.06	1.22
150 ♀	Glucose	78	40	172	132	849	112.3	36.1	15.3	342	7.29	2.18	1.37
151 ♀	Fructose	78	48	190	142	969	121.5	36.5	26.0	417	8.23	2.46	1.44
152 ♀	Glucose	78	47	197	150	969	131.1	40.3	16.7	376	8.15	2.40	1.57
153 ♀	Fructose	78	35	166	131	807	107.2	32.1	20.2	352	6.84	1.88	1.28
154 ♀	Glucose	78	35	180	145	807	115.2	36.3	23.0	389	7.30	2.13	1.37
155 ♀	Fructose	78	36	163	127	855	103.6	31.9	19.6	345	7.02	1.87	1.30
156 ♀	Glucose	78	36	174	138	855	115.9	35.1	15.9	335	6.84	1.92	1.25
157 ♂	Fructose	56	38	197	159	684	127.2	39.9	20.6	409	7.50	2.08	1.39
158 ♂	Glucose	56	39	213	174	684	141.0	41.8	20.8	429	7.23	2.04	1.41
Averages	Fructose	68.9	43.4	192.6	149.2	799.4	121.06	37.64	26.62	440.2	7.449	2.150	1.386
	Glucose	68.9	43.5	201.2	157.8	799.4	129.61	40.40	23.71	434.6	7.434	2.131	1.406

pairs of rats. The contents of ash, calcium and phosphorus were almost identical on the average for the glucose and fructose rats.

The percentage composition of the empty carcasses revealed interesting differences between the two groups of rats, as the averages and the statistical analyses summarized in table 5 testify.

On the percentage basis, the carcasses of the fructose rats were definitely, if only slightly, drier and fatter than those of the glucose rats, and less certainly they were richer in calcium and ash and poorer in protein. The average ratios of calcium

TABLE 5

A comparison of the percentage composition of the glucose and fructose rats

RATION	MOISTURE	PROTEIN	FAT	ASH	CALCIUM	PHOSPHORUS	ENERGY IN CALS./GM.
Fructose	62.80	19.51	13.82	3.90	1.126	0.725	2.282
Glucose	64.46	20.09	11.62	3.74	1.077	0.707	2.144
Statistical analysis of paired differences							
Mean [1]	−1.66	−0.579	+2.20	+0.162	+0.0486	+0.0176	0.139
Standard deviation	1.50	0.755	2.44	0.196	0.0614	0.0479	0.164
Probability	0.011	0.042	0.024	0.049	0.037	0.18	0.030

[1] A + sign indicates a greater average content in the fructose rats. A — sign indicates a greater average content in the glucose rats.

to phosphorus were 1.55 to 1 for the fructose rats and 1.52 to 1 for the glucose rats.

A digestion trial on all pairs of rats in this comparison, revealed a lower digestibility of the fructose ration. In seven of the pairs, the weight of dry feces formed on the same amount of food was greater on the fructose than on the glucose diet, the difference averaging 14.9%. In this case the probability of a chance outcome is 0.036. The weight of nitrogen in the fructose feces was greater in seven pairs than that in the glucose feces, but in this case the average difference is not significant statistically ($P = 0.063$). While the percentage of calcium in the dry feces was significantly greater on the glucose (6.86%) than on the fructose (6.09%) diet, with P

equal to only 0.011, the absolute amount of fecal calcium averaged higher on the fructose diet, although on the average the difference was slight and quite insignificant, P being 0.17. The apparent digestibility of the dietary energy was greater in seven of the eight pairs for the glucose diet, the average coefficients being 92.45 and 93.76. The difference in digestibility, though slight, was highly significant with $P = 0.016$.

In order to detect a possible differential effect of the carbohydrate components of the two experimental diets on the voluntary activity of the rats, all pairs were put into revolving cages equipped with revolution counters for periods of 43 to 56 days. In six of the eight pairs the glucose rat proved to be the more active, although the probability of a fortuitous outcome was so large (0.044) that it can hardly be neglected. Hence, the results of this test merely suggest that the glucose diet induced the greater activity.

Glucose versus lactose. The comparison of glucose and lactose (rations 4 and 5) was not particularly successful, except as it confirmed previous work to the effect that synthetic rations containing as high as 60% of lactose are unphysiological. The lactose rats soon became bloated and diarrheal. Their growth was slow and their appetite was poor. In one of the eight pairs started on test, the lactose rat died after 7 weeks of experimental feeding, during which no appreciable growth occurred. The results of the growth test and of the carcass analyses are presented in table 6.

The greater growth of the glucose rats in body weight and in all constituents of the carcass except calcium is clearly evident from these data. The results are unanimous among all pairs as regards gain in body weight and content of moisture, protein, fat, energy and ash. With respect to phosphorus, six pairs out of seven favor the glucose ration and the probability of a chance result is so small (0.023) that here also it may be concluded that, compared with glucose, lactose impaired dietary utilization. However, in three of the seven pairs, the calcium content in grams was greater for the lactose rat, and although the average was greater for the glucose rats, the difference between the two groups is clearly insignificant.

TABLE 6

The results of the comparison of glucose and lactose

RAT NO. AND SEX	SUGAR TESTED	DAYS ON TEST	INITIAL BODY WEIGHT	FINAL EMPTY WEIGHT	GAIN IN WEIGHT	TOTAL FOOD CONSUMED	COMPOSITION OF CARCASS						
							Moisture	Protein	Fat	Energy content	Ash	Calcium	Phosphorus
			gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*calories*	*gm.*	*gm.*	*gm.*
215 ♀	Lactose	46	45	63	18	202	44.7	11.7	2.46	89	3.13	0.92	0.58
216 ♀	Glucose	46	45	92	47	202	63.3	19.3	5.73	160	3.80	1.06	0.70
217 ♀	Lactose	56	44	92	48	316	64.5	17.7	4.27	153	4.39	1.28	0.81
218 ♀	Glucose	56	44	115	71	316	73.7	22.9	12.98	264	4.52	1.27	0.85
219 ♂	Lactose	60	47	127	80	403	88.0	25.5	6.49	217	5.61	1.59	1.08
220 ♂	Glucose	60	45	163	118	403	109.0	34.3	12.47	326	6.00	1.70	1.09
221 ♀	Lactose	56	43	105	62	333	71.7	21.5	6.47	181	4.83	1.42	0.91
222 ♀	Glucose	56	46	129	83	333	84.7	26.3	12.29	261	4.94	1.37	0.94
223 ♂	Lactose	56	28	86	58	288	60.7	16.6	3.98	137	3.54	0.97	0.67
224 ♂	Glucose	56	28	111	83	288	74.7	22.4	8.78	216	3.98	1.05	0.76
227 ♀	Lactose	54	36	47	11	194	35.5	8.4	2.28	54	2.78	0.86	0.51
228 ♀	Glucose	54	37	69	32	194	47.6	15.4	2.84	108	3.20	0.91	0.60
229 ♀	Lactose	37	73	109	36	241	75.8	21.5	5.82	176	4.77	1.36	0.90
230 ♀	Glucose	37	72	133	61	241	88.6	26.0	13.44	270	4.59	1.30	0.87
Averages	Lactose		45.1	89.9	44.7	282.4	62.99	17.56	4.539	143.9	4.150	1.200	0.780
	Glucose		45.3	116.0	70.7	282.4	77.37	23.80	9.790	229.3	4.433	1.237	0.830

The percentage composition of the carcasses reveals a somewhat different story, as the averages and statistical analyses in table 7 indicate.

On the basis of percentage composition, the lactose rats contained more water, less fat, energy and, less certainly, protein, but definitely more ash, calcium and phosphorus. The percentage of calcium in the lactose rats was far above normal and in one case, rat no. 227, reached the high value of 1.807. The average ratios of calcium to phosphorus were 1.56 to 1 for the lactose rats and 1.49 to 1 for the glucose rats.

TABLE 7

A comparison of the percentage composition of the glucose and lactose rats

RATION	MOISTURE	PROTEIN	FAT	ASH	CALCIUM	PHOSPHORUS	ENERGY IN CALS./GM.
Lactose	70.57	19.30	4.94	4.73	1.380	0.884	1.550
Glucose	66.94	20.65	8.12	3.89	1.085	0.731	1.944
Statistical analysis of paired differences							
Mean[1]	+3.63	−1.35	−3.18	+0.84	+0.295	+0.153	−0.394
Standard deviation	1.50	1.58	2.09	0.19	0.089	0.035	0.107
Probability	0.0005	0.042	0.0049	<0.0001	<0.0001	<0.0001	<0.0001

[1] A + sign indicates a greater average content in the lactose rats. A — sign indicates a greater average content in the glucose rats.

The digestion trial on five pairs of rats in the lactose-glucose comparison revealed a markedly impaired digestibility of the lactose ration. In all pairs the fecal dry matter and fecal energy were greater for the same food intake for the lactose rat. The fecal nitrogen determination was not successful for two of the glucose rats, but in the remaining three pairs, the fecal nitrogen was definitely greater on the lactose ration. On the contrary, the fecal calcium was smaller in amount in all five pairs for the lactose ration; on the percentage basis the dried lactose feces contained an average of only 3.11% calcium, while the dried glucose feces contained 6.14%. Surprisingly, the prevailing diarrheal condition induced by the lactose ration did not increase the fecal wastage of calcium.

The coefficients of apparent digestibility of energy were much larger in each pair for the glucose ration, the averages being 88.03 for the lactose ration and 94.30 for the glucose ration.

DISCUSSION

As compared with glucose, the reference sugar in all three comparisons, sucrose, fructose and lactose, induced greater losses of organic nutrients in the feces, as evidenced by significantly greater excretions of dry matter, nitrogen and energy in the feces for equal intakes of food. The food energy of the sucrose ration was 99.31% as digestible as that of the glucose ration with which it was compared, that of the fructose ration 98.60% as digestible, and that of the lactose ration 93.35% as digestible.

In the glucose-sucrose comparison the differences in digestible nutrients consumed by pair mates brought about by the difference in the carbohydrate components of the diets were so small in magnitude, even though great in statistical significance, that neither the rate of growth nor the total deposition of protein, fat or energy in the carcasses were significantly affected.

In the glucose-fructose comparison, the greater intake of digestible organic nutrients by the glucose rats induced a significantly greater rate of growth which was entirely accounted for by a greater deposition of water and protein in the tissues. From the data of the digestion trial, it may be computed that the glucose rats on the average consumed 496 mg. more of digestible nitrogen than did the fructose rats. Their carcasses contained on the average 440 mg. more nitrogen. The difference in intake of digestible energy, amounting to 42 calories in favor of the glucose rats, equivalent to 0.6 calorie per day, was evidently too small (Mitchell, '35) to induce a greater storage of energy in the carcasses of the glucose rats during the period of experimental feeding.

The greater impairment in the digestion of organic nutrients by lactose than by sucrose or fructose, was associated with much smaller gains in weight by the lactose rats than by

their pair mates on the glucose ration and with distinctly smaller depositions of organic nutrients in their bodies, Consuming 1.52 gm. less digestible nitrogen than their pair mates, the lactose rats deposited 1.01 gm. less nitrogen in their tissues. Consuming 72 calories less of digestible energy, they deposited 85 calories less energy in their carcasses.

While sucrose, fructose and lactose impaired to variable extents the digestibility of the organic nutrients in the experimental rations as compared in each case with glucose, they favored the utilization of calcium and, in the case of lactose, the utilization of phosphorus also. The sucrose rats deposited 7.6% more ash and 11.7% more calcium in their bodies than their pair mates receiving the glucose ration, and these differences were evident in eight of the nine pairs. The mineral content of the feces was not determined in this test.

In the glucose-fructose comparison, neither the deposition of ash, calcium and phosphorus in the bodies of pair mates, nor the excretion of calcium in the feces was significantly different. However, the percentage of calcium, but not of phosphorus, in the carcasses of the fructose rats was significantly higher than that in the carcasses of the glucose rats.

The lactose rats, growing at a rate equal to only 60% of that of their glucose controls, deposited 97% as much calcium and 94% as much phosphorus in their bodies. On an equal empty weight basis, their carcasses contained 28% more calcium and 21% more phosphorus than those of their pair mates. The losses of calcium in the feces were significantly greater on the glucose than on the lactose ration, the difference averaging about 10%.

In the glucose-fructose comparison, and particularly in the comparison of glucose and lactose, the utilization of calcium or of calcium and phosphorus in the non-glucose rats seemed to be limited by the slower growth occasioned by the impairment in the utilization of the organic nutrients. Indicative of this limitation is the greater percentage content of the non-glucose rat in calcium or in calcium and phosphorus both. In these cases, therefore, we may reasonably conclude that if the

intake of digestible organic nutrients had been the same for pair mates, the fructose and the lactose rats would have stored more calcium than their glucose controls, and the lactose rats would have stored more phosphorus.

SUMMARY AND CONCLUSIONS

When sucrose, fructose and lactose constitute 60 to 70% of the diets of growing rats, these diets being otherwise complete, the digestibility of the organic nutrients of the diets, as compared with that of diets containing equal concentrations of glucose, is distinctly impaired, the impairment being least for sucrose, intermediate for fructose, but by far the greatest for lactose.

The slight impairment in the digestibility of the organic nutrients in the sucrose ration did not affect the rate of gain of the rats nor the composition of the gains in such nutrients. On the fructose ration, the rate of growth was definitely slower as was the rate of deposition of protein. But on the lactose ration, the greatly impaired digestibility induced markedly slower rates of growth and rates of deposition of all organic nutrients.

The fecal wastage of calcium was not increased by fructose and lactose as compared with glucose, and the metabolic utilization of calcium was definitely increased by sucrose, fructose and lactose as compared in each case with glucose. Lactose, in addition, definitely promoted the utilization of phosphorus in metabolism as compared with glucose.

It may be concluded, therefore, that the differences in the metabolic disposal of the various sugars studied and their different physiological effects, observed when the sugars are administered alone, largely disappear when they are fed as components of a complete diet. Administered in a complete diet, the nutritive values of the sugars appear to be very much the same except for differential effects on the digestibility of the organic nutrients of the rations. Sucrose, fructose and lactose do seem to favor the utilization of calcium more than does glucose, while lactose promotes also the utilization of

phosphorus.[1] But these effects too may be the result of intestinal conditions more favorable to the absorption of calcium and of phosphorus prevailing on diets containing 60 to 70% of sucrose, fructose and lactose, as compared with those conditions prevailing on a predominantly glucose diet.

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[1] Some uncertainty may be felt in the justification for this conclusion on account of the calcium and phosphorus impurities probably existing in the lactose and fructose used. However, the sucrose effect, which was most likely produced by the contained fructose, cannot be so explained. This would indicate that the effects observed with fructose fed as such, were largely the result of the type of sugar rather than of any mineral contamination. The lactose effects on mineral metabolism are much larger than those observed with fructose, although the calcium contamination was only a third as great, and the phosphorus contamination, though greater, was still quite inconsiderable (0.0011%).

ACTIVATABILITY OF MILK AS AFFECTED BY FEEDING ERGOSTEROL TO COWS

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The absorption by animals of plant sterols, particularly ergosterol, has been studied by several investigators. Schönheimer and his associates ('29, '30) were not able to detect the absorption or storage of ergosterol in mice, rats, dogs, cats or rabbits. Yuasa ('29) demonstrated an increase in the sterol content of the portal blood of dogs following a meal rich in cholesterol, but was unable to show an increase when the plant sterol, sitosterol, was fed. Using laying hens, Schönheimer and Dam ('32) report an increase in the ergosterol content of the yolks of eggs laid by hens which were fed 50 mg. of ergosterol daily. The concentration of ergosterol in the yolk was increased from a normal of 0.0018% to 0.0027%. These studies indicate that the plant sterols are not absorbed by mammals in sufficient amounts to be detected chemically, but they are absorbed by birds in detectable amounts.

It was our purpose to determine whether the absorption of ergosterol could be demonstrated by biological methods and also whether the presence of ergosterol in milk increased its activatability. The latter question is of practical importance since the degree to which milk can be activated is limited, due to the development of an unpleasant taste and odor in the milk by prolonged irradiation.

PROCEDURE

The effect of the direct addition of ergosterol on the activatability of the milk was studied first. Ergosterol dissolved in

a mixture of 1 part chloroform and 4 parts propylene glycol was mixed into 5 gallons of milk to make up batch no. 1. A similar mixture without the added ergosterol constituted batch no. 2. The concentration of added ergosterol in batch no. 1 was 0.42 mg. per quart.

Each batch of milk was irradiated for 75 minutes by recirculation at the rate of 12 gallons per minute through an RUV machine equipped with a high pressure mercury vapor lamp operating at 170 V. Samples of each batch were assayed [1] with the following results:

TABLE 1

Tests of milks irradiated with and without added ergosterol

BATCH	DESCRIPTION	TEST LEVEL (USP UNITS PER QUART)	AVERAGE RESPONSES	
			Assay group	Reference group (2.7 USP units)
I	0.42 mg. added ergosterol per quart	1350	2.4+	2.2+
		2700	1.9+	2.3+
II	No added ergosterol	675	2.3+	2.2+
		1350	1.2+	2.3+

The data indicate that the presence of an added 0.42 mg. of ergosterol per quart of milk resulted in an increased activatability of the milk such that it acquired at least twice the antirachitic potency that ordinary milk attains with a comparable exposure to ultraviolet light. Under these conditions of irradiation the increased vitamin D potency amounted to more than 675 U.S.P. units per quart. A statistical examination of our own assay results, as well as the data given by Bills and associates ('31) and by Coward and Key ('33), indicates that

[1] The assays were made in accordance with the tentative procedure adopted by the A.O.A.C. (J. Assoc. Off. Agr. Chem., vol. 20, p. 79, 1937) using option no. 4. The supplements were mixed with sufficient amounts of the rachitogenic diet 2965 to last 7 or 8 days. The animals were killed on the eleventh day and line test readings made. The responses were rated in accordance with the table given by Bills et al. ('31), i.e., 2+ indicating a narrow continuous line. The reference assay groups received 2.7 units of the international standard. The supplements for the assay groups were calculated as follows:

$$2.7 \text{ (reference group level)} \times \frac{946 \text{ (cc./quart)}}{\text{Test level (assumed U.S.P. units/quart)}}$$

If the test level were 1350 U.S.P./quart then the supplement calculated by the formula above should amount to 1.89 cc. of the milk per rat.

vitamin D bioassays by the line test technic have a P.E. of approximately 15% when the assay groups consist of ten animals. Since our results indicate an increase of at least 100% in the potency attained by milk containing 0.42 mg. of ergosterol per quart over the control milk with comparable exposure, it should be possible by this method to demonstrate the presence of less than 0.2 mg. of ergosterol per quart, or about 1 part in 5,000,000.

In studying the second part of the problem, as to whether ergosterol when fed to cows is absorbed and secreted in the milk, three groups of three cows each, having approximately the same milk and butterfat production, were selected. These three groups were treated as follows:

Group I, control, regular ration.
Group II, regular ration + 4.3 ounces unirradiated yeast daily.
Group III, regular ration + 120 mg. unirradiated ergosterol daily.

After 2 weeks on the foregoing rations the milk production for 1 day was saved for irradiation. A 10-gallon aliquot was used for each group. The irradiation of each lot of milk was carried out in the RUV machine for 120 minutes by re-circulating at the rate of 12 gallons per minute.

These milks were tested at two levels. Each of the three milks was shown to contain more than 540 U.S.P. units of vitamin D per quart.

On retesting the milks at a 675 U.S.P. unit per quart level, the following results were obtained:

TABLE 2

Tests on irradiated milks

GROUP	DESCRIPTION	TEST LEVEL (USP UNITS PER QUART)	AVERAGE RESPONSES		AVERAGE BUTTERFAT CONTENT	AVERAGE MILK PRODUCTION
			Assay group	Reference group		
					%	*pounds*
I	Control	675	2.4+	1.9+	3.8	33.2
II	4.3 oz. yeast per cow per day	675	2.3+	2.0+	3.9	29.7
III	120 mg. ergosterol per cow per day	675	1.5+	2.0+	3.2	32.2

The experiments indicate that feeding 120 mg. of unirradiated ergosterol (either in the form of dry yeast or as crystalline ergosterol dissolved in oil) per cow per day did not increase the activatability of the milk produced.

After the foregoing feeding work was completed we transferred groups II and III from unirradiated material to irradiated yeast and irradiated ergosterol in oil, respectively. In both cases the cows received 325,000 U.S.P. units of vitamin D per cow per day. After these supplements had been fed for 3 weeks, samples of milk from a day's production were obtained for test. Table 3 gives the results of these tests.

TABLE 3

Tests on milks produced by feeding irradiated yeast and irradiated ergosterol

GROUP	SUPPLEMENT	TEST LEVEL (USP UNITS PER QUART)	AVERAGE RESPONSES	
			Assay group	Reference group
II	Irradiated yeast	485	1.6+	2.4+
		400	1.7+	2.0+
III	Irradiated ergosterol in oil	200	1.4+	2.4+
		135	2.0+	2.0+

The results obtained from feeding irradiated yeast and irradiated ergosterol confirm the previous work of Thomas and MacLeod ('31), Hess et al. ('31) and Russell et al. ('34), showing that the vitamin D fed in the form of irradiated yeast is from two to three times more effective in the production of vitamin D milk than the vitamin D fed as irradiated ergosterol in oil.

SUMMARY

The direct addition of ergosterol to milk increased the activatability of the milk. Addition of 0.42 mg. of ergosterol per quart resulted in the milk attaining more than twice the antirachitic potency of normal milk when the two were given comparable exposures to ultraviolet light.

Feeding ergosterol (dissolved in oil or given in yeast) to cows did not result in an increased activatability of the milk on exposure of the milk to ultraviolet light. This result is in

accord with reported chemical studies which indicate that plant sterols are not absorbed by mammals.

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ON THE SURVIVAL OF THE COMPLETELY DEPANCREATIZED DOG[1]

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Since the discovery of insulin, several reports dealing with the survival of the depancreatized dog receiving insulin have appeared. The early reports indicated a failure to effect survival beyond 7 or 8 months (Allan, Bowie, Macleod and Robinson, '24). Later, by the addition of raw pancreas to the diet, Macleod ('30) effected the survival of two dogs for as long as 4 years after excision of the pancreas. A progress report from this laboratory (Chaikoff, '35) showed that with a suitable diet survival for as long as 5 years was effected in two dogs despite the complete absence of raw pancreas from the diet. Recently, however, Dragstedt and his co-workers ('36) have again reported failure in effecting survivals beyond a few months unless raw pancreas or a fraction of this is added to the diet. In view of these differences of experience, a careful study was undertaken in this laboratory of the length of survival of the completely depancreatized dog maintained with insulin and a diet adequate in calories, proteins, salts and vitamins, but containing no pancreas.

EXPERIMENTAL

All dogs to be depancreatized were carefully selected. For several weeks after arrival at the laboratory, they were fed a

[1] Aided by a grant from the Breon Fund of the University of California Medical School, San Francisco. The insulin was generously donated by Eli Lilly and Company. The assistance rendered by the Works Progress Administration is also gratefully acknowledged.

high-calorie, high-vitamin diet, and only those which—besides being normal in all respects— showed a vigorous appetite for the standardized diet that is fed depancreatized dogs in this laboratory were selected for operation.

Following pancreatectomy each animal received 8 units of insulin twice daily, at 8 A.M. and 4 P.M. Just before each injection of insulin, they were fed the following mixture:

	gm.
Raw lean beef	280 [2]
Sucrose	50
Bone ash	7

Vitamin supplements (A and D as cod liver oil; [3] the B complex in the form of a concentrate obtained from rice bran [4]) were added to the diet mixture twice each week, each animal receiving about 25 cc. of cod liver oil and 15 cc. of the rice bran concentrate in that period. This concentrate contained about 50 international units of B(B_1) per cubic centimeter and 10 modified [5] Bourquin-Sherman units of G (flavin) per cubic centimeter. The same concentrate has also been shown to be a good source of both rat and chick antidermatitis factors (Lepkovsky, Jukes and Krause, '36).

The animals were kept in large cages having metal screen bottoms. At no time during the entire stay in the laboratory were they exposed to extremes of temperature.

During the first week or two after pancreatectomy, the animals showed a diminished appetite, but soon regained a vigorous appetite. In order to avoid chronic undernutrition, animals that failed to regain a good appetite within a month or so after pancreatectomy were discarded. In all animals recorded in this study, the diet mixture was completely ingested

[2] This diet was adopted in September, 1935. Previously the animals had received 225 gm. of lean beef and 70 gm. of sucrose, as well as the other constituents listed.

[3] The standardized cod liver oil used in this study was kindly furnished by Mead Johnson and Company.

[4] The rice-bran concentrate was kindly furnished by Vitab Products, Inc., Emeryville, California.

[5] The basal diet for rat assay was modified to supply the rat and chick antidermatitis factors. We are indebted to Mrs. M. K. Dimick of the Vitab Products laboratory for the assays of this material.

TABLE 1

The maintenance of completely depancreatized[1] dogs for periods longer than 1 year with diets containing neither raw pancreas nor choline supplements[2]

DOG	PRE-OPERATIVE WEIGHT	WEIGHT AT END OF PERIOD OF MAINTENANCE	PERIOD OF MAINTENANCE FOLLOWING PANCREATECTOMY	REMARKS
	kg.	*kg.*	*years*	
DA♀	9.5	7.5	5.5	Sacrificed while in good condition
DC♀	11.9	10.7	5.1	Sacrificed while in good condition
DB♂	7.3	7.0	4.2	Sacrificed while in good condition
G8♀	9.0	7.1	3.3	Sacrificed while in good condition
DF♀	10.1	9.4	3.1	Met with accidental death. In good condition at time of accident
DD♂	8.3	7.4	2.8	Survival observations on this diet terminated. Animals in good condition at end of this interval
K♂	11.0	6.5	2.7	Died. Bronchopneumonia and acute pyelitis found at autopsy. Refused food for several weeks before death
DE♀	8.1	6.6	2.3	Survival observations on this diet terminated. Animal in good condition at end of this interval
G1♀	8.0	6.7	1.8	Sacrificed while in good condition
G3♀	8.0	4.5	1.8	Died. Emaciated at time of death despite vigorous appetite throughout[3]
G2♀	10.4	8.0	1.7	Died. Infected hydronephrosis with multiple small abscesses in right kidney found at autopsy
DJ♀	8.9	7.2	1.6	Died. Extensive retroperitoneal hemorrhage and cellulitis with involvement of heart found at autopsy
A3♀	9.3	8.8	1.6	Sacrificed while in good condition
DG♀	8.3	6.7	1.5	Died. Acute urinary tract infection found at autopsy. Refused food for 2 weeks prior to death
MF♀	9.6	8.5	1.3	Sacrificed while in good condition
A1♀	8.8	7.3	1.3	Sacrificed while in good condition

[1] Completeness of pancreatectomy confirmed in all cases at necropsy.

[2] It should be noted that the diets employed were not choline-free. The term 'supplements' refers to choline in addition to that already present in the high-calorie, high-protein, high-vitamin diet fed in this laboratory.

[3] The degree of emaciation shown by this dog was not observed in other animals. Such variations might be the result of variations in the degree of absorption to be found after pancreatectomy (McClure, Vincent and Pratt, '17).

within a few minutes after it was served. On occasions a few of the animals showed a temporary loss of appetite, but this rarely lasted longer than 1 or 2 days.

All animals were subjected to a careful search for pancreatic tissue at necropsy.[6] The completeness of all pancreatectomies recorded in tables 1 and 2 was confirmed.

RESULTS

Table 1 records the observations on dogs maintained between 1 and 5.5 years, table 2 those on the dogs that remained in this laboratory for periods less than 1 year.

TABLE 2

Summarized results of completely depancreatized[1] dogs maintained for less than 1 year

PERIOD OF MAINTENANCE AFTER PANCREATECTOMY	NUMBER OF DOGS	SACRIFICED OR TRANSFERRED TO OTHER EXPERIMENTS AT END OF MAINTENANCE PERIOD WHILE IN GOOD CONDITION	NUMBER THAT DIED DURING INTERVAL STUDIED
months			
7–12	7	7	0
6– 6.9	3	3	0
5– 5.9	6	5	1[2]
4– 4.9	12	12	0
3– 3.9	14	13	1[3]

[1] Completeness of pancreatectomy confirmed at necropsy.
[2] Death associated with loss of weight, jaundice and bile in urine.
[3] Did not finish meals completely. Died in hypoglycemia.

Sixteen animals were maintained here for periods between 1.3 and 5.5 years after excision of all pancreatic tissue (table 1). They may be grouped as follows:

1. Eight dogs were maintained for 1.3 to 1.8 years. Four of these were removed from the survival study while in good nutritional state and, to all external appearances, normal. Two of the eight dogs died suddenly; at autopsy one showed a hydronephrosis of the right kidney; in the other, an extensive infection was found in many tissues.

[6] We are indebted to Dr. C. L. Connor and Dr. G. R. Biskind of the Division of Pathology for examination of all tissues in this study.

2. Five dogs survived for periods between 2.3 and 3.3 years. Four of these were in good shape at the time the survival study was terminated. Dog K suddenly refused food and died shortly thereafter.

3. Dogs DA, DB and DC are specially significant in that their periods of survival are the longest so far recorded for completely depancreatized dogs maintained on a diet adequate in all respects but containing no pancreas. All animals were active and possessed a vigorous appetite at the time the survival periods were terminated for examination of their tissues. By external appearance they could not be distinguished from normal dogs.

The forty-two dogs recorded in table 2 were kept in the laboratory for periods less than 1 year and, with the exception of two animals, all were in good shape at the time they were sacrificed.

DISCUSSION

The results of the present study show quite clearly that survival for as long as 4 or 5 years is possible in the completely depancreatized dog so long as he is supplied with insulin and a diet containing large amounts of those constituents known to be essential for the maintenance of a normal dog under optimal conditions, namely proteins, vitamins and salts. In view of the impaired absorption resulting from excision of the gland, the use of a high-calorie, high-protein, high-vitamin diet such as the one used in this study is necessary for prolonged survival. Moreover, since the present purpose was to investigate to the fullest extent possible the effects of the excision of the pancreas without the complications induced by undernutrition, only those animals—with few exceptions—were retained that showed a vigorous appetite for the diet employed.

Since the survival periods recorded here are the longest so far reported, it may be concluded that raw pancreas or other

dietary supplements such as choline [7] are not necessary for the survival of the completely depancreatized dog receiving insulin.

It should be emphasized at this point that despite their survival for these long periods, completely depancreatized dogs manifest certain abnormalities when maintained under the conditions of this study. Indeed, it has been shown in this laboratory that fattiness of the liver is not the only pathological change encountered in the depancreatized dog receiving no raw pancreas but an otherwise adequate diet. The following are among the other changes noted:

1. Cataracts appear as early as 1 year after pancreatectomy (Chaikoff and Lachman, '33).

2. The blood lipid constituents, in particular cholesterol, undergo marked alterations (Chaikoff and Kaplan, '34). These changes may appear as early as 3 weeks after pancreatectomy.

3. Although fatty livers appear early and remain for long periods following excision of the gland, a regression in the fat content of this organ occurs if the animals survive long enough (Kaplan and Chaikoff, '37). This regression is associated with a marked proliferation of fibrous connective tissue arising around the portal triads. In three dogs that survived from 4 to 5.5 years, the livers showed extensive periportal fibrosis with irregular lobulation indicative of cirrhosis.

It may be concluded that in a large number of cases these pathological changes need not interfere with survival so long as the diet is adequate. The alterations in the lipid content of blood and liver observed under these conditions, however,

[7] While it is here shown that choline supplements are not necessary for survival, it should not be inferred that the choline content of the diet is without significance in this regard. The stock diet employed in this laboratory supplied approximately 400 mg. of choline daily. To prove whether choline is an essential constituent for the survival of the completely depancreatized dog treated with insulin would necessitate the use of a diet adequate in all other respects (i.e., calories, proteins, salts and vitamins) but free from choline. As yet no such experiment has been conducted on depancreatized dogs.

are not the result of a deficient supply of insulin, for it has been shown in a series of completely depancreatized dogs recorded elsewhere (Kaplan and Chaikoff, '37) that so long as pancreas is added to the stock diet and its feeding begun immediately after pancreatectomy, the amount of insulin administered, namely 16 units daily, not only keeps the liver lipids at normal levels but also permits the maintenance of a body weight that is either normal (preoperative) or well above the normal.

Although a search at necropsy revealed a complete absence of pancreatic tissue in all animals recorded in this study, the following data are of interest regarding the state of these dogs. The sugar level of the blood and the excretion of glucose in the urine were repeatedly examined during their stay in the laboratory. The following blood sugars taken in the postabsorptive state [8] are representative: Dog DA, 384 mg. per cent; dog DB, 259; dog DC, 272; dog DD, 358; dog DE, 417; dog DF, 476. Glucose, in varying amounts, was always present in the 24-hour sample of urine. When the insulin was decreased or completely withdrawn, an immediate increase in the output of both glucose and nitrogen occurred, and this was followed by a loss of weight and an inability to maintain a positive nitrogen balance.

Previous attempts at survival of the completely depancreatized dog in the absence of raw pancreas and choline supplements.[9] A number of cases have been cited as evidence against lengthy survival, and it has been maintained that supplements either of pancreas (or a fraction thereof) or of choline [9] are necessary for survival beyond a few months. An examination of the diets employed, however, reveals that in some cases they were inadequate in one or more respects and that the importance of feeding a diet high in vitamins and protein to

[8] Blood was taken for analysis between 8 and 9 A.M.; the dogs had received their last injection of insulin and their last meal at 4 P.M. of the previous day. This state of the animal, in which it has been deprived of both food and insulin for 16 hours, is here referred to as the postabsorptive state.

[9] As noted above, 'choline supplements' refer to choline other than that contained in the dietary constituents.

balance the loss of pancreatic enzymes was not always recognized.

1. Allen, Bowie, Macleod and Robinson ('24). The diet employed consisted solely of meat and sucrose. It need not be stressed that this diet is so inadequate in vitamins and salts that early death is by no means surprising.

2. In Fisher's work ('24) no mention is made of the diet employed or of the use of vitamins.

3. Bliss ('22) worked with a single dog that was very weak and emaciated before pancreatectomy. The diet consisted of a ground hash of bread and cooked meat moistened with hot soup; occasionally milk was also given.

4. In the more carefully conducted work of Hershey and Soskin ('31), vitamin deficiencies were recognized and reported. Hershey and Soskin obtained survivals for as long as 11 months without the aid of raw pancreas or lecithin supplements.

5. Penau and Simonnet ('26) report survival of several completely depancreatized dogs for periods over 2 years with diets containing no pancreas. Their animals were fed at frequent intervals a diet containing boiled beef, fresh milk, bread, sugar and bone ash.

6. Dragstedt et al. ('36) state that their completely depancreatized dogs fail to survive more than a few months. In view of the foregoing observations, such a short period of survival is indeed surprising. Each of Dragstedt's animals received daily a diet consisting of 400 gm. of meat, 400 cc. of whole milk and 100 gm. of white bread. All these constituents are poor sources of vitamin B(B_1) (Cowgill, '34; Gunderson and Steenbock, '32; Leong and Harris, '37; Roscoe, '31; Samuels and Koch, '32; Sure, '33). It is possible that the vitamin B(B_1) content of the diet is on the borderline of the minimum requirements for the normal dog. This diet is low, moreover, in vitamin D, and although it may approximate the requirements of this constituent for the adult dog, it is probably not ideal for a growing animal.

Because of the tendency to pathological changes in the liver and other tissues, it is by no means unlikely that an adequate supply of vitamins, proteins and salts is of greater importance in the maintenance of the depancreatized dog than in that of the normal animal. A diet meeting the minimum requirements of a normal dog may be seriously deficient for an animal suffering from impaired absorption. Dragstedt and coworkers ('36) report that their animals suffered from loss of appetite, loss of weight, apathy and muscular weakness. These symptoms are similar to those associated with vitamin B deficiency in the dog, namely anorexia, loss of weight and loss of control of the limbs (Cowgill, '34). It is also interesting to note that convulsive seizures are not uncommon occurrences in dogs subsisting on a diet deficient in vitamin B_1. The importance of providing liberal amounts of vitamins for dogs deprived of pancreatic juice was previously pointed out by Handelsman, Golden and Pratt ('34).

It must now be apparent that in most cases where complete failure in survival was reported due consideration was not given to the vitamin content of the diet. Even with the diet employed in this laboratory, a loss of weight usually occurs after pancreatectomy (compare table 1). It is obvious, therefore, that the caloric intake is not excessive; nor is too much protein given. Indeed, in view of the impaired digestion it is advisable to give large amounts of protein. The importance of avoiding protein undernutrition by providing an adequate protein intake becomes all the more significant from the showing that diets low in choline but high in fat do not produce fatty livers in rats receiving a good amount of protein (Channon and Wilkinson, '35; Best, Grant and Ridout, '36).

While—in view of the impaired absorption—a diet at least adequate in calories, proteins, vitamins and salts is obviously a minimum requirement for survival, the pathological changes that appear in these animals show quite clearly that survival does not occur under optimum conditions. In regard to one of these changes, namely the fatty liver, it has already been shown that this can be prevented or impeded by the addition

of pancreas, either raw or autoclaved, to the diet (Kaplan and Chaikoff, '37). Choline has also been shown to be effective in preventing the deposition of fat in the liver (Best, Ferguson and Hershey, '33). Approximately 400 mg. of choline are provided daily by the stock diet employed in this study. The relation of this amount of choline and of other possible limiting factors to survival and pathological changes is at present under investigation.

SUMMARY

1. When maintained with insulin and a high-calorie, high-protein, high-vitamin diet, completely depancreatized dogs may survive for as long as 4 to 5 years. The pathological changes observed in these animals during survival are recorded.

2. This length of survival makes it unnecessary to assume that raw pancreas or extracts thereof or choline supplements (i.e., in addition to that contained in the dietary constituents) are essential for the survival of the completely depancreatized dog receiving insulin.

3. The previous failures to obtain lengthy survival of completely depancreatized dogs maintained with insulin and a diet containing no pancreas are examined and shown to be of questionable significance in view of the inadequate diets employed.

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ENVIRONMENTAL TEMPERATURE AS A FACTOR IN THE PRODUCTION AND IN THE CURE OF RICKETS IN THE RAT [1, 2]

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ONE FIGURE

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In the past, nutritional investigators have experienced what appeared to be irregular variations in the antirachitic potency of certain vitamin D preparations. These variations in antirachitic potency have now become recognized as variations in the antirachitic sensitivity of the test animals, and have become more generally apparent since the adoption of the U.S.P. technic for the assay of vitamin D and the subsequent distribution of the reference cod liver oil as a standard of potency. The routine use of definite amounts of this reference standard at monthly intervals offers an excellent opportunity to detect such variations in antirachitic sensitivity. Since these assays are usually carried out according to a carefully standardized procedure, these variations in biological response must result from factors other than those usually considered in the assay technic.

Many investigators have attempted to eliminate these variations in antirachitic sensitivity by observing certain precautions in the feeding and in the care of their breeding colony. Some have considered such factors as size and age of the test

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[2] Presented before the division of biological chemistry at the Chapel Hill meeting of the American Chemical Society, April 12 to 15, 1937.

animals when placed on the rachitogenic diet. Others have considered the problem from the standpoint of environmental factors such as the temperature and the humidity of the environment. In fact, Tourtellotte and Bacon ('35) have presented data from which they have drawn the conclusion that variations in the sensitivity of the rachitic test animals are due to fluctuations in laboratory temperature during the rachitic and test periods. These investigators intimate that high environmental temperature predisposes to short depletion periods and low biological potencies.

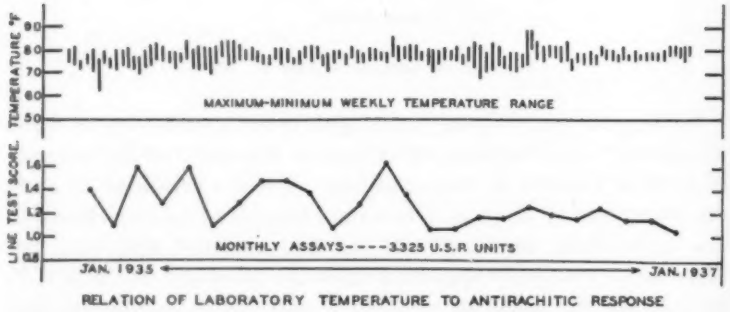

Fig. 1 Showing the relation of the variations in laboratory temperature to the antirachitic potency of the U.S.P. reference cod liver oil as determined by monthly assays during the years of 1935–1936.

If such physical factors as temperature and humidity should be found to be the principal causes of these variations in biological response, this condition might be eliminated once and for all by properly air conditioning the biological laboratory. However, the data available in our laboratory when the above article appeared, failed to show that any uniform relationship existed between the variable sensitivity of our test animals to rickets and the temperature of the experimental laboratory (fig. 1). Realizing, however, that our data had been obtained under a somewhat restricted range of temperatures, we felt that it was not a true index of the influence of temperature on the above condition. It seemed to us that the effect of en-

vironmental temperature on the biological response of rachitic animals could be determined most accurately by conducting simultaneous tests at accurately controlled temperatures, using uniform test animals and maintaining all other factors comparable. The following pages describe such a study.

EXPERIMENTAL

Since most biological laboratories are maintained at temperatures approximating 80°F., we decided to maintain our control animals at this temperature. For the extreme, we chose temperatures approximating 70° and 100°F., which we believed to be sufficiently wide to cover the most practical operating conditions. In order to maintain these temperatures under thermostatic control, a special cabinet was constructed of insulating material around one of our regular three-tier laboratory cage units. The three compartments, each containing one tier of cages, were insulated from each other by a double layer of the insulating material. The surfaces of the compartments were reinforced by wooden framework. In order to facilitate the cleaning and the care of the experimental animals, all compartments were provided with a door on each side. Each compartment, containing eight individual cages, was equipped with heating or cooling elements, thermostatic controls, and other necessary equipment to maintain their temperature at the desired level. For the top and the middle compartments, electrical heating elements were used. The temperature of the bottom compartment was maintained by means of a copper coil through which a regulated amount of cold water flowed. This coil was mounted against the top of the compartment and under it was hung a drip-pan to catch and carry away condensed water. When the necessary equipment had been assembled and put through some preliminary tests, it was found that the copper coil in the bottom compartment was not sufficiently large to reduce the temperature of that compartment to 70°F. under all seasonal conditions. It would, however, maintain a temperature of 73 ± 1°F. Because of this fact, the temperature of the

middle compartment was adjusted to 84° instead of 80° as previously planned. The experimental equipment, therefore, when placed in operation consisted of three compartments, each of which contained eight individually caged animals that were maintained at temperatures of approximately (±1°F.) 73, 84 and 100°F., respectively.

This investigation consisted of two parts: first, to determine the effect of temperature on the production of rickets, and secondly, to determine the effect of temperature on the recalcification of rachitic rats when fed a definite unitage of vitamin D. In the first of these studies, two series of animals were used, while in the second phase four regular series were found to be necessary and, in addition, several control groups. All test animals were of the same strain, purchased from the same source and were of approximately the same age when placed on experiment. In placing the animals on experiment, unusual care was exercised to select only animals of the same sex and of the same weight for the three compartments. All animals were fed liberal quantities of the rachitogenic diet (Steenbock and Black no. 2965, '25) and were supplied with clean distilled water at all times. In fact, the care and handling was the same as that accorded our regular vitamin D assay animals. Animals used in the recalcification studies were given 2 U.S.P. units of vitamin D on the first and again on the fourth day of the 10-day curative period. The temperatures of the three compartments were carefully recorded each day at a regular hour. The right hind leg of each animal was x-rayed on the eighteenth and the twenty-first (last) day of the depletion period and again on the seventh and the tenth (last) day of the curative period. At the end of the experiments all animals were anesthetized with ether, the blood removed by heart puncture, the tibiae removed for bone ash determinations, and the femora for 'line tests.' The inorganic calcium and the inorganic phosphorus of the serum of the combined blood from all animals in each compartment was determined by the Clark-Collip ('25) modification of the Kramer-Tisdall ('21) method and by the

Youngburg et al. ('30) method. The flesh-free tibiae were dried, extracted in a Soxhlet extractor with ethyl alcohol for a period of 12 hours and again extracted for a like period with sulfuric ether. The extracted bones were again dried, weighed and ashed at a red heat until constant weight resulted.

DATA

The data obtained in these studies have been condensed in tabular form and are presented in the following table. Owing to the close similarity between the x-ray findings among the groups of each series, it was found impossible to express the stages of calcification in terms of a quantitative measure; therefore, no data obtained by this criterion are included. The x-rays were of considerable value, however, in following the progress of decalcification during the depletion period. The relative degrees of decalcification manifested by the negative control animals at the end of the depletion period were evaluated in terms of an arbitrary scale in which conditions approximating mild, moderate and severe rickets were expressed as -1, -2 and -3, respectively. The degrees of recalcification as indicated by the 'line test' were evaluated in the usual manner in which a value of 2 represented a thin but continuous line of darkened granules appearing on the metaphyseal side of the epiphyseal cartilage. Greater or lesser degrees of recalcification were designated accordingly. The following data represent the average values of all animals of the respective groups.

DISCUSSION

Since the above experiments were carried out during a period extending from late winter to early fall, the test animals used should have been fairly representative of those commonly employed in biological assays so far as the seasons of the year are concerned. With every experimental series, unusual efforts were made to select only vigorous animals of the same sex and of the same weight for each of

TABLE 1

Showing the average values obtained from the various groups of animals during the investigation. These values have been tabulated in the order of increased environmental temperature and also according to the three stages of calcification represented

GROUP NO.	NUMBER OF ANIMALS	AVERAGE TEMPERATURE OF COMPARTMENT	AVERAGE INITIAL WEIGHT	DEPLETION PERIOD	CURATIVE PERIOD	UNITS VITAMIN D FED	AVERAGE FINAL WEIGHT	AVERAGE DAILY FOOD INTAKE	AVERAGE 'LINE TEST' SCORE	SERUM		AVERAGE BONE ASH
										Ca per 100 ml.	P per 100 ml.	
Negative controls—rachitogenic diet without supplement												
		°F.	*gm.*	*days*	*days*	*U.S.P.*	*gm.*	*gm.*	*− or +*	*mg.*	*mg.*	*%*
1-A	8	74	45	21	0	0	65	5.7	−1.9	10.4	3.3	36.6
2-A	6	72	45	21	0	0	61	5.1	−1.8	11.1	4.5	36.7
1-B	8	84	45	21	0	0	62	5.0	−1.8	12.3	3.5	38.8
2-B	8	84	44	21	0	0	60	4.6	−2.0	11.3	4.3	38.4
7-B	8	84	43	31	0	0	70	4.9	−2.5	10.1	2.9	29.8
1-C	7	101	45	21	0	0	58	3.6	−2.0	10.8	3.2	37.7
2-C	4	100	43	21	0	0	56	3.4	−2.0	12.3	4.6	40.3
Experimental—rachitogenic diet plus vitamin D supplement												
3-A	5	73	43	21	10	4	67	6.5	+1.6	9.5	6.3	32.8
4-A	8	72	45	21	10	4	67	6.3	+1.5	10.7	9.2	43.5
5-A	7	73	46	21	10	4	70	5.8	+1.9	10.7	5.2	34.3
6-A[1]	6	72	44	21	10	4	64	5.9	+1.8	12.7	7.7	36.4
3-B	8	84	45	21	10	4	66	5.6	+1.3	12.6	6.7	34.5
4-B	7	85	45	21	10	4	68	5.5	+1.3	10.8	8.4	41.2
5-B	8	84	46	21	10	4	72	5.0	+1.9	12.8	4.9	32.7
6-B[1]	8	84	44	21	10	4	61	4.7	+1.5	12.5	5.0	36.0
3-C	(All animals over heated)											
4-C	7	100	45	21	10	4	66	3.9	+1.7	10.5	6.8	43.6
5-C	6	101	46	21	10	4	61	3.1	+1.2	11.7	4.9	34.7
6-C[1]	6	100	44	21	10	4	56	3.2	+1.7	12.4	6.2	36.7
Positive controls—receiving breeding colony diet												
8-B	8	84	44	2 days at 84°F.			48	6.9	Normal	11.3	10.1	51.7
9-B	6	84	43	6 days at 84°F.			60	8.2	Normal	11.7	9.3	52.2
10-B	8	84	45	10 days at 84°F.			71	9.6	Normal	11.4	9.4	53.5

[1] All animals composing these groups were females. All other animals used in the investigation were males.

the three compartments. In fact, the method of selection was so exacting that in some series the individual weights of all animals of the three compartments, when placed on the rachitogenic diet, did not differ by more than 3 gm. All animals used were males except those of the sixth series, which were all females.

For the sake of reference, the three experimental compartments were designated from bottom to top as compartments A, B and C, respectively. As previously stated, the approximate temperatures at which these compartments were maintained were 73, 84 and 100°F., respectively. The various series of animals used were designated numerically in chronological order. A reference to a particular group, for example group 5-B, denotes, therefore, the fifth group of animals maintained in compartment B at a temperature approximating 84°F. Other groups will be similarly designated.

The relation of environmental temperature to the daily food intake of the various test animals became distinctly evident during the first series of studies and remained equally evident throughout the entire investigation. As was to be expected, those animals which were subjected to the lower temperatures consumed greater quantities of the rachitogenic diet. In fact, at the end of the depletion period those groups of animals which were maintained at temperatures of 84 and 73° had consumed, on an average, 13 and 31% more food, respectively, than had similar animals maintained at 100°F. When the experiments were continued over a 10-day curative period, these differences in food consumption increased to 50 and 82%, respectively. The average increase in body weight was also greater at the lower temperatures. This would indicate that, under the conditions of the investigation, the rat's requirement for food was not restricted to that required for energy production plus the additional amount required to produce a uniform rate of growth. The relative amounts of food required to produce a unit gain in body weight at the temperatures studied were: 5.9, 5.1 and

[1] All animals composing these groups were females. All other animals used in the investigation were males.

4.5 gm., respectively, at the end of the depletion period and 8.7, 7.5 and 6.6 gm., respectively, at the end of the curative period.

The degree of rickets manifested at the end of the depletion period, as indicated by the line test, showed no significant differences that might be in any way attributed to differences in environmental temperature. There was a very slight indication that more severe rickets were produced at the higher temperature, but it appears doubtful if the method of scoring the rachitic condition was sufficiently sensitive to make such differences significant.

The line test score as determined at the end of the curative period did not indicate that environmental temperature is an important consideration in recalcification in the rat. The average values of the scores do indicate slightly greater recalcification at the lower temperatures, but differences among these values are less than differences among line test values obtained from different groups of animals maintained at the same temperature and otherwise treated identically.

Those animals which had received the rachitogenic diet during a 21-day depletion period likewise failed to show that environmental temperature had any definite effect on serum calcium, serum phosphorus or bone ash. Here again groups of animals of different series which had been maintained at the same environmental temperature showed as much variation in these values as did groups of animals of the same series which had been maintained at different temperatures. At the end of the curative period, however, there were indications that a higher environmental temperature resulted in a higher serum calcium and a lower serum phosphorus. Here again group differences were marked and it appears doubtful if the apparent temperature effects were significant.

A point which appears worthy of mention concerns the relative mortality among the groups of animals maintained at the different temperatures. It may also be well to state that the third group of animals (group 3-C) maintained at the higher temperature was accidentally subjected to an increased tem-

perature (108°F.) during one night of the second week of the experiment, due to the improper functioning of the thermostat. As a result of this increased temperature, a number of the animals died during the night and others were so severely weakened that the entire group was discarded. Other than this group, the total mortalities numbered a total of twenty-seven, of which eight occurred at the lowest temperature, one at the medium temperature and eighteen at the highest temperature. Nothing was observed, however, that would indicate that the higher mortality was in any way related to a more severe rachitic condition.

We were somewhat surprised to find that those animals which had been maintained at the lowest temperature and which had consumed the greatest amount of food, and in consequence had made the greatest gain in body weight, did not show a significant difference in the degree of rickets manifested. In our regular vitamin D assays, where all animals are maintained at a comparable temperature, those animals which make the greatest increase in body weight invariably show the highest degree of rickets.

A general consideration of these data leads us to conclude that environmental temperatures, within the ranges studied, are rather unimportant considerations in either the production or the cure of rickets in the rat. On the other hand, the data do indicate that the history of the test animal previous to being placed on the rachitogenic diet is a worth while consideration. This is well illustrated by the animals comprising the fourth series (groups 4-A, 4-B and 4-C) which showed considerably higher serum phosphorus and bone ash values at all temperatures than did the animals of any of the other series. Such variations in test animals appear to be independent of the body weight when placed on experiment. It is highly probable that this can be at least partially attributed to unrecognized variations in the breeding colony diet.

SUMMARY

A series of studies have been made concerning the effect of environmental temperature on the production and on the cure of rickets in the rat. Comparable experiments were carried out at temperatures of 73, 84 and 100°F., using a uniform rachitogenic diet and carefully selected test animals. The stages of calcification were measured by means of x-rays, line test, bone ash, and serum calcium and phosphorus.

The data obtained in these studies indicate that environmental temperature is not an important consideration in either the production or the cure of rickets in the rat. In fact, undetectable differences in different groups of rats when placed on the rachitogenic diet led to greater variations in experimental results than did a variation of approximately 28°F. in environmental temperature.

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FURTHER STUDIES ON THE EFFECT OF EXCESSIVE VITAMIN A ON THE OESTROUS CYCLE OF THE RAT

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Baumann and Steenbock ('32), Mason and Ellison ('35) showed the effect of avitaminosis A; Sherwood, Brend and Roper ('36) showed the effect of hypervitaminosis A on the vaginal smear of rats. It was therefore deemed advisable in the present investigation to study the effect of excessive vitamin A administration upon the oestrous cycle. A study of the vaginal smear, the uterine and ovarian picture was made to show this phenomenon. The procedure of this investigation was designed to show if possible, the mechanism responsible for these changes.

EXPERIMENTAL

Vaginal smears were obtained on a group of twenty-six rats approximately 150 days of age. From four to thirteen oestrous cycles were observed for the purpose of determining the length of the normal cycle in these animals. Fifteen of the rats were then injected daily with 1500 international units of carotene [1] for a period of 5 to 15 days. Eleven animals were fed 1500 international units of carotene daily for a period of 5 to 32 days. The smears were taken on all animals during the period of vitamin A administration.

Five of the animals were killed after the carotene had been administered 5 to 10 days. Sixteen animals were killed at

[1] The carotene used in the present investigation was generously furnished by S.M.A. Corporation, Cleveland. Ohio.

intervals after carotene had been administered 11 to 32 days. Five rats were allowed to return to the normal oestrous cycle following carotene administration.

In order to prevent a cyclic disturbance due to an upset in nutrition and environment, the animals were kept in a room of constant temperature and given a well-balanced stock diet (Purina chow).

TABLE 1

The effect of carotene injections upon the vaginal smear, uterus and ovary

RAT NO.	NORMAL CYCLES	AVERAGE CYCLE	DAYS OF THERAPY	ABNORMAL	UTERUS	OVARY	ANIMAL KILLED
		days		days			
1	10	4.4	5	3	Proestrus 3.0 mm.	Follicles med. and large	5th day
2	13	4.2	7	5	Proestrus 2.3 mm.	Follicles med. and C.L.	7th day
3	13	4.1	10	8	Proestrus 2.7 mm.	Follicles large	10th day
4	8	4.6	10	8	Diœstrus 2.2 mm.	C.L. large	10th day
5	11	4.5	12	10	Diœstrus 2.0 mm.	C.L. large	12th day
6	12	4.2	12	10	Proestrus 2.7 mm.	Follicles small C.L.	12th day
7	12	4.3	15	16	Proestrus 2.9 mm.	Follicles small C.L.	18th day
8	8	4.1	15	16	Proestrus 2.7 mm.	Follicles small C.L.	18th day
9	7	3.9	15	16	Diœstrus 1.7 mm.	Follicles small C.L.	18th day
10	12	4.4	15	15	Proestrus 3.1 mm.	Follicles large C.L.	18th day
11	7	4.2	15	15			18th day
12	7	4.5	15	15	Diœstrus 1.7 mm.	Follicles vary C.L.	18th day
13	8	4.3	15	36			Returned to normal
14	6	4.4	15	33			Returned to normal
15	6	4.4	15	33			Returned to normal

RESULTS

The control animals that had received cottonseed oil showed no upset in the oestrous cycle. The animals that had received carotene continued to show a vaginal smear picture of leucocytes and nucleated epithelial cells during the period of carotene therapy. The injected rats rarely showed cornified

TABLE 2

The effect of carotene feeding upon the vaginal smear, uterus and ovary

RAT NO.	NORMAL CYCLES	AVERAGE CYCLE	DAYS OF THERAPY	ABNORMAL	UTERUS	OVARY	ANIMAL KILLED
		days		*days*			
16	10	4.7	5	3	Proestrus 2.5 mm.	Follicles small C.L.	5th day
17	6	3.8	11	8	Dioestrus 2.1 mm.	Follicles small C.L.	11th day
18	4	4.7	11	9	Dioestrus 2.5 mm.	Follicles vary C.L.	11th day
19	6	4.1	13	11	Proestrus 5.0 mm.	Follicles large C.L.	13th day
20	6	4.1	15	12	Proestrus 4.0 mm.		15th day
21	6	4.1	29	27	Dioestrus 1.7 mm.	Follicles vary C.L.	29th day
22	4	4.7	29	27	Oestrus 6.0 mm.	Follicles large C.L.	29th day
23	5	4.7	29	27	Proestrus 4.0 mm.	Follicles med. and C.L.	29th day
24	6	3.8	32	30	Dioestrus 1.7 mm.	Follicles small C.L.	32nd day
25	8	4.5	15	34			Returned to normal
26	4	5.2	15	17			Returned to normal

cells and the fed animals presented slightly greater numbers of cornified cells in the vaginal smear. In no case were the cornified cells typical of oestrus.

The average length of the oestrous cycle was determined from the control series obtained preceding the carotene administration. Vaginal smear records were obtained approximately 10 minutes prior to the death of each animal.

These smears were compared with the expected normal smears that should have been obtained had the animals not received carotene.

Table 3 presents the data obtained from the vaginal smear studies. Thirty to 80 hours would have been required for the animals to reach the calculated stage as determined from the control series. Some variation existed in the length of time for the various cycles in the control series. However,

TABLE 3

The effect of vitamin A upon the oestrous cycle of the albino rat

RAT NO.	AVERAGE CYCLE	VAGINAL SMEAR			CAROTENE THERAPY	SMEAR VARIED
		Beginning of injection	Day killed	Expected smear		
Carotene injected						
	days				*days*	*hours*
1	4.4	Cor++[1] L+[1]	L+ Ne++[1]	Cor++ L+	5	61
2	4.2	Cor+++	Ne++ Cor+	L+++	7	45
3	4.1	L+ Ne++	Ne++ Cor+	Cor++ L+	10	35
4	4.6	Cor++ L+	L+ Ne++	Cor++ L+	10	55
5	4.5	L+ Ne++	L+ Ne++	L+++	12	40
6	4.2	L+ Ne++	L+ Ne++	L+++	12	30
7	4.3	L++ Ne+	L++ Ne+	L++ Ne+	17	00
8	4.1	L+ Ne++	L++ Ne+	Ne++ Cor+	17	80
Carotene fed						
20	4.1	Cor+ L++	Ne+++	L+++	13	55
21	4.1	L++ Ne+	L++ Ne+	Cor+++	28	70
23	4.7	Cor+ L++	Ne++ Cor+	Cor++ L+	28	50
24	3.8	L++ Ne+	L++ Ne+	Ne++ Cor+	31	37

[1] The abbreviations are to designate the presence of cornified, leucocytes and nucleated epithelial cells and the plus sign indicates the cell type in the majority.

normal oestrous cycles were never observed during the carotene administration. After the second day of carotene therapy, all animals showed great numbers of nucleated epithelial cells and leucocytes. The majority of animals gave no indication of going through the cornified cell stage. Several animals were not sacrificed and showed normal cycles approximately 20 days following the cessation of carotene administration.

In the study of the uteri as shown in tables 1 and 2, it was found that only five of the twenty-six animals demonstrated

uteri above 3.0 mm. in diameter, the majority of them being apparently in late dioestrus and early proestrus. The ovaries presented a similar picture. There were few large follicles and many corpora lutea in various stages.

DISCUSSION

Since Baumann and Steenbock ('32) reported that excessive cornification, in the vaginal epithelium of the albino rat, was an early manifestation of vitamin A deficiency, and were able to produce dioestrous smears within 1 week and oestrus in 2 weeks after the administration of carotene, it was thought advisable to study the effect of excessive vitamin A since the vitamin has a decided effect upon epithelial cells.

Mason and Ellison ('35) reported a marked increase in cornified cells in avitaminosis A. This effect may have been an indication that the epithelial cells died early and that the body was not able to remove the cornified cells as rapidly as produced. One might have expected therefore a normal or even an excessive amount of vitamin A to have kept these cells in a healthy condition and have prevented cornification.

The excessive vitamin A administration in the present investigation apparently produced an upset in the oestrous cycle as shown from the study of the uteri and ovaries as well as the vaginal smears. Since the control rats gave no indication of a changed vaginal smear picture, it would seem possible that the vitamin A was responsible for an upset of the oestrous cycle, or brought about marked mitosis, thus producing greater numbers of nucleated epithelial cells.

The animals that had received carotene by subcutaneous injection may have shown a more marked effect due to the fact that the vitamin was more efficiently utilized because of a slow absorption and no loss through the alimentary canal.

Further studies on the changed uterine picture are being made for the purpose of checking more adequately the vitamin A effect. The possibility of a hormone-vitamin relationship is also being investigated.

SUMMARY

Vitamin A given in the form of carotene orally and subcutaneously produced a change in the oestrous cycle of the albino rat. The vaginal smears showed nucleated epithelial cells and leucocytes during the entire period of carotene therapy. Very few cornified cells were observed during the experimental period.

Vitamin A given subcutaneously produced a greater effect upon the vaginal smear and uterine pictures than did oral administration.

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THE RESPIRATORY METABOLISM OF RATS RECEIVING A DIET DEFICIENT IN INORGANIC CONSTITUENTS. THE CHANGE IN BASAL METABOLISM [1]

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ONE FIGURE

(Received for publication May 6, 1937)

The field of respiratory metabolism in relation to mineral deficiencies is largely unexplored, although considerable knowledge is available regarding many effects of such deficiencies which are indicative of respiratory metabolic disturbances. Aside from some rather conflicting reports on the influence of calcium and phosphorus deficiencies there are no data on record, insofar as the writers are aware, which are directly concerned with the respiratory metabolism in relation to mineral deficiencies.

In an investigation of the influence of calcium deficiency on the basal metabolism of rats, Pedotti ('21) observed that after feeding a calcium deficient diet to young rats for a period of 3 weeks the fasting metabolic rate of the subjects was considerably decreased as compared with the fasting metabolism determined prior to this dietary treatment, although the irrita-

[1] The data reported in this paper were taken from a dissertation presented by Max Kriss in partial fulfillment of the requirements for the degree of Doctor of Philosophy, Yale University, 1936.

A preliminary report was presented before the division of biological chemistry, American Chemical Society, eighty-ninth meeting, New York, April, 1935.

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bility of the rats was increased. Subsequent feeding of a diet adequate in calcium resulted in a slight increase in the fasting metabolism, but failed to bring it to the pre-experimental level. The author concluded that calcium deficiency has the effect of lowering the basal metabolism. The possible influences of change in age and of the plane of preliminary feeding on the basal metabolic rate were not considered. The respiratory measurements were made at temperatures close to 20°C., which is much below the critical temperature for the rat.

Riddell, Hughes and Fitch ('33) measured the oxygen consumption of lactating cows kept alternately at normal and at low phosphorus intake. The measurements were made by means of a portable metabolism apparatus in 6-minute periods 12 hours after feeding. The authors observed that the phosphorus deficiency resulted in an increased oxygen consumption indicating an increased rate of metabolism. Kleiber, Goss and Guilbert ('36) determined the respiratory exchange of phosphorus-deficient beef heifers in the fasting condition and also after the ingestion of food. They found that the phosphorus deficiency had no effect on the fasting katabolism, but that it decreased the efficiency of energy utilization.

Observations on the influence of sodium deficiency on the utilization of energy and protein, which have an important bearing on the general subject of the present investigation, were reported by Kahlenberg, Black and Forbes ('37). In a paired-feeding experiment, rats receiving a diet deficient in sodium, stored less energy as fat and protein in their bodies and produced more heat, as compared with rats receiving an adequate supply of sodium. The heat loss was calculated from the analyses of the food, excreta and the rats' bodies.

A possible relationship between mineral deficiency and respiratory metabolism is suggested by the observations on the metabolic, hemopoietic and structural changes resulting from the strict limitation of inorganic salts in the diet (Swanson and Smith, '32, '34; Brooke and Smith, '33; Clarke, Bassin and Smith, '36; Smith and Smith, '34 a; '34 b; Eppright and Smith, '37 a, '37 b). Smith and Smith ('34 a) made the ob-

servation that although rats receiving a low-salt diet gained less in weight in comparison with their calorie controls, their voluntary activity and insensible weight loss, which normally are an index of metabolic intensity, decreased rather than increased. These findings raised a question as to the compensatory mechanism whereby the mineral deficient animal disposes of its available energy supply in bringing about the apparently lowered economy of food utilization. The following possibilities suggested themselves:

The mineral deficiency might have the effect of increasing the basal energy requirements of the animal; if the basal metabolism were unaffected, the specific dynamic effect of the mineral deficient diet might be so altered as to cause an increase in the total metabolism; the processes of digestion and absorption might be disturbed to such an extent as to lower the economy of food utilization; the mode of heat disposal might be so altered by the lack of mineral salts in the diet as to affect the relationship between the metabolism and the insensible weight loss.

The present paper is a record of experiments designed to elucidate the influence of the low-salt diet on the basal metabolism.

EXPERIMENTAL

Twenty male albino rats of the Connecticut Agricultural Experiment Station strain were used in this investigation. Only those were selected which weighed 40 gm. or more at weaning (21 days). This prerequisite was arbitrarily imposed with a view of securing a more uniform rate of growth during the period immediately following weaning, which is designated as the pre-experimental period. During this period the rats were fed an adequate stock diet ad libitum and housed in individual metal cages.

The pre-experimental feeding periods were of 12 to 17 days' duration, terminating when the rats had attained a weight of approximately 120 gm. Food was then withdrawn for 24 hours, after which the rats were subjected to an 8-hour meta-

bolism test. The chief purpose of this initial test was to secure a base line from which to view the results of the metabolic studies conducted after the imposition of the experimental dietary regime.

After the completion of the first basal metabolism test the rats were placed on purified diets by pairs. One rat of each pair received a ration which, exclusive of vitamin supplements, was extremely low in inorganic salts; it was composed of specially washed casein, 18%, dextrin, 55%, and hydrogenated fat, 27%. This diet is designated diet I, or the low-salt diet, and provided 0.075% total ash. Taking into account the voluntarily restricted food intake of the animals consuming the salt-poor ration, Smith and Smith ('34 b) calculated that the following amounts of the inorganic constituents per rat per day were eaten: phosphorus 14.6 mg., total base 0.320 m.eq., calcium 0.013 m.eq., magnesium 0.018 m.eq., potassium 0.228 m.eq., sodium 0.044 m.eq., chloride 0.072 m.eq. The other rat of the pair, a litter-mate, which served as the control, received in addition to diet I, 4% of a satisfactory salt mixture (Osborne and Mendel, '19). This ration containing the mineral salts is designated diet II. All rats received the same vitamin supplements. These consisted of dried yeast (200 mg. per day), alcoholic extract of wheat germ (1 cc. per day, equivalent to 2 gm. of wheat embryo) and 6 drops of cod liver oil per day. Food was offered twice daily in two equal portions, the feeding being controlled by the paired-feeding technique.

The rats were kept on the experimental rations for a period of approximately 3 months. Some of the rats were subjected to basal metabolism measurements at approximately monthly intervals, but all rats which survived were subjected to a final basal metabolism test at the end of the experimental feeding period. All experimental work was conducted, as far as it was possible, with both rats of each pair simultaneously.

The basal metabolism measurements started 24 hours after food and, with very few exceptions, continued for 8 consecutive hours. Measurements of carbon dioxide production were

made at hourly intervals, while determinations of oxygen consumption and of respiratory quotients were made for the entire experimental periods.

The respiration measurements were made by means of an open-circuit respiration apparatus similar in construction to that recently described by Forbes, Kriss and Miller ('34). Certain modifications were introduced, of which the most important were the use of a constant temperature air bath instead of a water bath, the provision for the visibility of the rats at all times by means of reflecting mirrors, and the abandonment of the wire screen cylinder as a means of restricting the movement of the animal. Bright illumination alone sufficed to accomplish the latter purpose.

The respiration chamber consisted of a 1-quart fruit jar 8 inches high and 4 inches in diameter, provided with a screw lid of standard size through which two pieces of copper tubing were fitted which served for connection to the air circuit. An ordinary jar rubber gasket was used to make the connection between the lid and the jar air-tight. Inside the jar was placed a strip of ½-inch galvanized wire screen about 2½ inches in width which served as a platform for the rat, thus preventing it from coming in contact with any excreta voided during the experiment. This screen was held in place by a loop of steel wire which was soldered to it. The size of the jar and the wire screen in relation to the size of the animals used were such that the animals could move about without difficulty.

Uniform conditions of temperature, ventilation and light were maintained throughout these experiments. The temperature of the air bath into which the chambers were placed was maintained close to 28°C. and the rate of ventilation was approximately 60 liters per hour. For illumination one 15-watt electric bulb was placed in the center of the lid of the air bath above each of the two respiration chambers. Under these conditions the activity of the rats was greatly restricted without any signs of discomfort. Close observations of the activity of the animals while in the respiration chamber were useful in reducing the respiration measurements to a basis of comparable inactivity.

The schedule of the respiration experiments is presented in table 1.

TABLE 1

Schedule of experiments

RAT NOS.		AGE	DAYS ON DIET	RAT NOS.		AGE	DAYS ON DIET	RAT NOS.		AGE	DAYS ON DIET
Low salt	Control			Low salt	Control			Low salt	Control		
1 and	2	35	..	11 and	12	129	91 and 92	23 and	24	36	..
..[1]	2	91	56	13 and	14	32	..	23 and	24	67	31
3 and	4	36	..	..[2]	14	119	87	23 and	24	93	57
3 and	4	123	87	15 and	16	34	..	23 and	24	119	83
6 and	7	34	..	15 and	16	109	75	25 and	26	39	..
6 and	7	123	89	15 and	16	118	84	25 and	26	68	29
9 and	12	37	..	19 and	20	33	..	25 and	26	94	55
11 and	10	38	..	19	..[3]	118	85	25 and	26	122	83
9 and	10	125	88 and 87								

[1] Rat 1 died 12/21/34 as a result of salt deficiency.

[2] Rat 13 died 2/9/35 as a result of salt deficiency.

[3] Rat 20 died 2/28/35 as a result of an accident.

RESULTS

The influence of activity

The measurements of the carbon dioxide production at hourly intervals, instead of for the total experimental period, together with the direct observations of the animal, have yielded important information regarding the fluctuations of the metabolism as affected by activity. The carbon dioxide production during the first hour was invariably high. This was associated with the activity which the rat exhibited during the early part of the first hour. It took in most cases from 3 to 5 minutes to weigh the animal with the jar tightly closed. During this time the rat was never entirely quiet. It also required some time for the rat to become adjusted to the environmental conditions after the respiration chamber was connected with the air circuit. After the first or second hour, however, the hourly CO_2 production was relatively uniform. In all cases the subject was found inactive during several consecutive hours. The number of hours during which the rat exhibited no activity varied in different periods. In some cases as many as six or even seven of the eight hourly periods of observation represented periods of inactivity.

The desirability of eliminating effects of activity of the subject from determinations of basal metabolism is obvious. To this end, in our final computations, we have excluded from the average, those results obtained during hours of activity. It was of considerable interest, however, to know to what extent the activity actually influenced the metabolism under our experimental conditions, and to determine what effect, if any, the mineral deficiency had in this respect. For this purpose, we have calculated the average hourly heat production of all the rats in all periods on two bases: 1) including the hours of activity and 2) excluding the hours of activity. The results obtained in the final fasting periods, following the 83 to 92 days of the low-salt feeding, are compared in table 2.

TABLE 2

The average hourly heat production during the final series of experiments, including and excluding hours of activity

RAT NO.	DIET NO.	AVERAGE HOURLY HEAT PRODUCTION		NUMBER OF HOURS AVERAGED IN (2)	DIFFERENCE $\frac{(1)-(2)}{(2)}$
		Including hours of activity (1)	Excluding hours of activity (2)		
		cal.	*cal.*		%
3	I	820	760	5	7.9
4	II	729	659	6	10.6
6	I	757	743	7	1.9
7	II	726	683	6	6.3
9	I	1008[1]	821	4	
10	II	798	736	5	8.4
11	I	836	748	4	11.8
12	II	812	696	3	16.7
14	II	869	757	4	14.8
15	I	718	677	5	6.1
16	II	787	689	5	14.2
19	I	784	740	5	5.9
23	I	775	723	5	7.2
24	II	783	622	3	25.9
25	I	776	700	4	10.9
26	II	695	631	5	10.1
Average	I (low salt)	781	739		7.4
Average	II (control)	774	684		13.4

[1] This high value is due to an abnormal condition of the animal and was not included in the average.

In the initial fasting periods, that is, before the rats were subjected to the purified diets, the differences between the average hourly heat production as calculated on the two bases ranged from 1.8% (with rat 2) to 9.4% (with rat 25), the average difference of all twenty comparisons being 5.4%. In this series of experiments the number of hours which represented periods of inactivity ranged from 4 to 7. In the majority of cases these periods of inactivity were represented by 5 or 6 hours. The same was true in the intermediate fasting experiments. In the latter the increases in the average hourly heat production resulting from the inclusion of the hours of activity were, with two exceptions, less than 10%. The average increase in metabolism with the low-salt animals (5.5%) was, however, considerably less than the average increase (11.4%) with the controls.

In the final fasting periods after 83 to 92 days on the salt-poor diet (table 2), the activity appears to have exerted a considerably larger effect than in either the initial or intermediate periods. Here the differences ranged from 1.9% (with rat 6) to as high as 25.9% (with rat 24). With one exception (rat 25) the differences in heat production due to activity were much less with the low-salt animal than with the control. The average difference for the low-salt animals was 7.4%, as compared with 13.4% for the controls. Indeed, it was observed that the low-salt animals were less active and appeared to be more relaxed than were the controls. In case of the salt-deficient rat 9 the large difference in heat production between the average of 4 hours of apparent inactivity and the average of all 8 hours is due to an abnormal condition of the animal. During the last 4 hours of observation the rat was not moving, but was breathing unusually fast and exhibited slight tremor, and the CO_2 production rose abnormally high. This rat died the following day.

The influence of the mineral deficiency on the basal metabolism

Accepting the general definition of basal metabolism as the metabolism measured under conditions of muscular repose

and when no food is being digested or absorbed, we have utilized for the determination of the basal metabolism of the rats the data representing the average hourly heat production of the fasting animals, excluding the hours of activity. For

TABLE 3

Influence of mineral-poor ration on basal metabolism

RAT NO.	BEFORE GIVING EXPERIMENTAL DIETS			DIET NO.	AFTER MAINTENANCE ON EXPERIMENTAL DIETS				DECREASE (1)−(2) / (1)
	Initial body weight	Calories [1]			Final body weight	Days on diet	Calories [1]		
		Per 100 gm. per hour	Per M^2 per 24 hours (1)				Per 100 gm. per hour	Per M^2 per 24 hours (2)	
	gm.	*cal.*	*cal.*		*gm.*		*cal.*	*cal.*	%
3	108	0.744	911	I	147	87	0.587	718	21.1
4	110	0.711	870	II	139	87	0.527	645	25.9
6	110	0.756	925	I	145	89	0.580	710	23.3
7	109	0.689	843	II	165	89	0.489	599	29.0
9	107	0.721	882	I	162	88	0.595	728	17.5
10	103	0.735	900	II	155	88	0.550	673	25.2
11	103	0.733	987	I	157	91	0.554	678	24.4
12	102	0.686	840	II	191	92	0.452	553	34.1
13	111	0.715	875	I					
14	104	0.724	886	II	190	87	0.428	524	40.9
15	103	0.767	939	I	111	84	0.631	772	17.7
16	110	0.738	903	II	157	84	0.510	624	30.9
19	111	0.724	886	I	153	85	0.557	682	23.1
20	102	0.717	878	II					
23	111	0.770	942	I	138	83	0.601	736	21.9
24	108	0.749	917	II	151	83	0.466	570	37.8
25	115	0.717	878	I	159	83	0.534	654	25.5
26	108	0.741	907	II	157	83	0.465	569	37.2
Average		0.739	914	I (low salt)			0.580	710	21.8
Average		0.721	882	II (control)			0.486	595	32.6

[1] Kilogram-calories.

the purpose of comparison we have expressed the results on the basis of 100 gm. body weight per hour, as well as per square meter of body surface per 24 hours. These data for the initial and final fasting periods are presented in table 3.

The computation of the metabolism to a basis of 100 gm. was made not in direct proportion of the body weight but in proportion to the two-thirds power of the body weight. In this manner recognition was given to the conception of an exponential relationship between energy metabolism and body weight.

The computation of the results per square meter of body surface was made on the basis of Meeh's formula, $S = 9.1\ W^{\frac{2}{3}}$, which was used by Benedict and MacLeod ('29 a, b) and adopted by Horst, Mendel and Benedict ('30, '34 a, b, c, d). The latter calculation was made not without the realization of the uncertainty of the determination of the surface area of the rat. The inaccuracies involved in the computation of the metabolism per square meter of body surface have been discussed by Benedict ('31–'32), Kleiber ('32) and Brody ('34).

Before the rats were placed on the experimental diets their basal metabolism was over 700 gram-calories per 100 gm. per hour and about 900 kilogram-calories per square meter per 24 hours, the results being fairly uniform. The mean of all individual determinations was found to be 730 calories per hour or 898 kilogram-calories per square meter per 24 hours, with an average deviation from the mean of $\pm 2.3\%$.

The respiratory quotients were also very uniform. The average of all determinations in the initial period was found to be 0.739 ± 0.005.

Contributing to this uniformity of the results are the facts that the animals were of the same strain, of the same sex, of approximately the same age and weight, and that they had been all kept on the same diet prior to this initial fasting period. The fact that the values were practically free from effects of activity is perhaps the most important factor in this relationship.

At the end of approximately 3 months (83 to 92 days) on the experimental diets a marked influence of the mineral deficiency on the basal metabolism is shown by the comparison of the results for the low-salt animals with those obtained with the controls. Without a single exception, the basal meta-

bolism of the salt-deficient animal was found to be considerably higher than the value for the control. The average basal metabolism of eight salt-deficient animals which survived, was found to be 710 calories per square meter per 24 hours as compared with 595 calories for the controls. The difference of 115 calories represents an increase in favor of the salt-deficient group of 19.3%. These results are highly significant and indicate that the elevated basal metabolic rate of the animals which received the low-salt diet was brought about by the mineral deficiency.

Very little or no effect was observed on the fasting respiratory quotients, since the final respiratory quotients with both groups of animals were identical, or nearly so, with those obtained in the initial fasting periods. This may be interpreted to signify that the effect of the mineral deficiency on the basal metabolism was only in respect to the intensity.

The influence of advancing age and suppression of growth

When the basal metabolism of the animals in the final fasting periods is compared with the metabolism in the initial periods (table 3, last column), it is observed that a marked reduction in the basal metabolic rate occurred with both groups of animals. This general decline in the basal metabolism, per unit of weight or size, represents the combined effect of advance in age and of the limited food intake. The effect of the mineral deficiency was to render this decline less pronounced. With the salt-deficient animals the decreases in basal metabolism ranged from 17.5% to 25.5%, averaging 21.8%, whereas with the controls these decreases ranged from 25.2% to 40.9% and averaged 32.6%.

The results obtained with rats 23, 24, 25 and 26, which are presented graphically in figure 1, are of especial interest in that they show the changes in the basal metabolism at approximately monthly intervals. These results show that the decline in metabolism was most pronounced during the first month and that it was significantly greater with the controls than with the low-salt animals. The basal metabolism of rat 23

(low-salt) declined during the first 31 days on the experimental diet from 942 calories per square meter per 24 hours to 783 calories—a decrease of 16.9%. During the same interval the decline in metabolism with rat 24 (control) was from 917 calories to 685 calories per square meter—a decrease of 25.3%. During the next 52 days the basal metabolism of rat 23 declined only 5.0% of the initial value, while the decline with rat 24 was 12.5%. Similarly, the basal metabolic rates

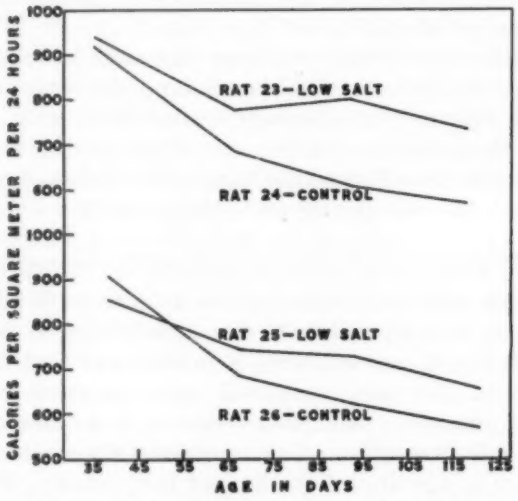

Fig. 1 The influence of advancing age and suppression of growth on the basal metabolism of rats receiving the low-salt diet, and of their controls.

of rat 25 (low-salt) and rat 26 (control) declined, respectively, 14.9% and 24.2% during the first 31 days, and 10.6% and 13.0% during the next 52 days.

It is clear from figure 1 that the response of the rats to the mineral deficient diet, insofar as it affects their basal metabolic rate, is relatively prompt. It is a significant fact that the greatest divergence between the growth curves of eight of the ten pairs of rats compared, occurred during the first month of the experimental dietary treatment, the low-salt animal

making the smaller gains in weight. The coincident rise, during this period, in the basal energy requirements of the animals receiving the low-salt diet, as compared with their controls, may serve to explain, to a large extent, the failure of the salt-deficient animals to utilize their diet for body increase as efficiently as did the controls.

DISCUSSION

It is a pertinent question to ask what the immediate cause for the increased basal metabolism of the salt-deficient rat is. We can give no direct answer to this question; as will be shown in a subsequent paper, the protein metabolism cannot account for it. The rectal temperature of the low-salt rats is consistently lower than that of their controls.[4] The increased metabolism of the former cannot, therefore, be directly attributed to body temperature. Brody ('32) has suggested that the basal metabolism of rats is proportional to the size of the visceral organs. Since some of the visceral organs, notably the kidney, of the low-salt rats were found to be enlarged (Swanson, Storvick and Smith, '36), this enlargement of the organs may be one of the causes for the increased basal metabolism.

In view, however, of the finding (Swanson and Smith, '32) that the low-salt diet brings about a condition of polycythemia with a reduction of the hemoglobin content of the blood—a constituent which plays a profound role in the processes of respiration—it seems more probable that the physico-chemical changes which have been found to take place in the blood as a result of the mineral deficiency are directly responsible for the marked alteration in the basal energy transformations of the animal. The very recent finding (Eppright, '36) that certain endocrine organs, notably the adrenals, are affected by the low-salt diet suggests the possibility that certain hormones are involved. This is a problem for future investigation.

[4] Unpublished observation of Dr. P. K. Smith.

SUMMARY AND CONCLUSIONS

The basal metabolism of young rats kept on a low-salt diet, as well as that of their calorie controls receiving an adequate supply of mineral salts, declined with increasing age during a period of approximately 3 months. The decline was most pronounced during the first month and was greater with the controls than with the low-salt animals. The rats kept on the mineral deficient diet were less active and invariably had a considerably higher basal metabolism than their controls. The salt deficiency apparently had no effect on the respiratory quotients of the fasting rats, which may be interpreted to indicate that the effect on the metabolism was only in relation to the intensity.

The authors express their appreciation to Dr. James Melville for valuable technical assistance in connection with this investigation.

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VITAMINS A, C AND D IN MAIZE AS AFFECTED BY VARIETY AND STAGE OF GROWTH [1, 2]

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This investigation was carried on to determine the effect of variety and stage of growth on the content of vitamins A, C and D in maize plants and kernels when grown under similar field conditions. Evidence is also presented to show that the comparative vitamin content of plant material is subject to a wide variation depending upon whether it is expressed on a moist or a dry basis.

The vitamin A content of maize kernels has been investigated by Fraps ('31), Hauge ('30), Mangelsdorf and Fraps ('31), Russell ('30), Steenbock and Boutwell ('20), Takahashi and Masuda ('35) and others; all of whom have found yellow maize superior to white maize in vitamin A content. The vitamin A content appears to vary directly with the number of genes for yellow pigmentation in the endosperm.

Ten varieties of maize: Canada Flint, Rhode Island Flint, Country Gentleman, Lancaster Sure Crop, Bantam Evergreen, Rustler's White, Golden Bantam, Black Mexican, Golden Sunshine and Stowell's Evergreen were grown in experimental plots on Merrimac fine sandy loam soil with 2000 pounds of 5-8-7- fertilizer mixture per acre. When the plants were approximately 25 cm. high a representative sample of the entire plant, except for the roots, was taken of the following varieties: Canada Flint, Rhode Island Flint, Country Gentleman,

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[2] Presented before the ninety-ninth meeting of the American Association for the Advancement of Science at Atlantic City, N. J., December 31, 1936.

Lancaster Sure Crop, Bantam Evergreen and Rustler's White. Later in the summer when the plants had attained their maximum growth and the kernels were at the milk stage, representative samples of the above six varieties were again taken and the percentage dry matter and vitamin A and C contents determined. The final samples were taken 1 month later, at which time the leaves and stalks had begun to turn brown. Fully or partially formed ears were not included in these plant samples. The vitamin A and moisture content of all the samples were determined. Vitamin C determinations were made on the first two series of samples. These data are presented in tables 1 and 2.

The percentage dry matter in each sample was determined by drying at 100 to 105°C. for 24 hours. The vitamin A content of each sample was determined by the Sherman and Munsell bio-assay method (Sherman and Smith, '31).

The rats previously depleted of vitamin A were used in series of eight at the several feeding levels for each variety. The samples of young corn plant were harvested each day and fed fresh over a 7-day period, then the samples were solidly frozen and used as needed for the rest of the feeding period. The other corn samples were frozen immediately after harvesting. Freezing and freezing storage are not known to injure vitamin A.

The immature kernels were fed fresh for 7 days and then sufficient was frozen for the remainder of the assay period. The mature kernels were dried and stored on the cob for 3½ months. The kernels were then ground to pass a 20-mesh sieve and kept in glass-stoppered bottles during the period of assay.

Vitamin C was determined by the 2, 6-dichlorophenolindophenol dye method of Tillmans as described by Bessey and King ('33), but modified by using for the extraction and acid solution 1 N with respect to sulfuric acid and N/4 with respect to metaphosphoric acid according to Mack, Tressler and Dearborn ('36). Comparisons made with the guinea pig assay method on immature sweet corn showed the chemical method to be reasonably accurate.

Vitamin D was determined by the usual U.S.P. ('34) assay method using Reference cod liver oil as a standard. Fresh plants and immature kernels were used in all but one series of the vitamin D assays. The exception consisted of corn plants which had been harvested and solidly frozen for 4 days before they were fed. This is explained in table 4.

EXPERIMENTAL RESULTS

From table 1 it is evident that there was a definite increase in the vitamin A and dry matter content of maize plants as they developed up to the full grown stage. There appears to be

TABLE 1

Effect of maturity of maize plants on vitamin A and moisture content

VARIETY	COLOR	DRY MATTER			VITAMIN A PER GRAM MOIST BASIS			VITAMIN A PER GRAM DRY BASIS			CONDITION OF MATURE PLANT
		Young	Full grown	Mature	Young	Full grown	Mature	Young	Full grown	Mature	
		%	%	%	*I.U.*	*I.U.*	*I.U.*	*I.U.*	*I.U.*	*I.U.*	
Canada Flint	Yellow	10.6	17.8	25.3	5.9	15.9	8.7	55.7	89.4	33.9	Green
Lancaster Sure Crop	Yellow	9.8	16.9	30.2	4.9	12.8	10.5	50.0	75.8	34.6	Green
Bantam Evergreen	Yellow	10.0	19.0	34.6	4.4	17.1	3.3	44.0	90.0	9.2	Dry
Rhode Island Flint	White	10.6	18.0	33.9	7.0	12.6	2.6	66.2	70.0	7.5	Dry
Country Gentleman	White	13.7	17.7	29.9	4.0	12.2	16.1	29.2	69.0	53.1	Green
Rustler's White	White	11.0	15.6	52.3	5.8	10.8	1.7	52.2	62.5	3.3	Dry
Average		10.9	17.5	34.3	5.3	13.5	7.1	49.5	76.0	23.6	

no definite correlation between the vitamin A content of the young maize plant and the amount of yellow color in the mature kernel. However, in the full grown plants, those bearing yellow kernels had a slightly higher vitamin A content. The higher vitamin A content of the full grown plants is actual and is not merely an apparent increase due to an increase in dry matter. The change in vitamin A content as the plant matures is much greater when calculated on a moist basis rather than on a dry basis. The mature plants showed a marked decrease in vitamin A content and an increase in dry

matter over the full grown plants. In the mature plants, however, the vitamin A content varied directly with the degree of greenness. The sample of Rustler's White which was very dry, showed a very marked loss of vitamin A. The variety highest in vitamin A content when young was Rhode Island Flint; when full grown, Bantam Evergreen was highest and Rustler's White was lowest.

The rapid loss of vitamin A in maize plants after they have attained maximum growth and tend to dry out is an important factor which should be taken into consideration when such material is used as a feed for livestock, either fresh or as silage. It is recommended that maize, when used for such purposes, should be harvested as soon as its full growth has been reached, in order to conserve its vitamin A content.

When the kernels of the Golden Bantam, Bantam Evergreen, Golden Sunshine, Country Gentleman, Stowell's Evergreen and Black Mexican were at the milk stage, (eating stage for the sweet corn varieties) and again after full maturity samples were taken and assayed for vitamins A and C. As shown in table 3, only the yellow varieties contained significant amounts of vitamin A. The three well-known sweet corn varieties, Golden Bantam, Bantam Evergreen and Golden Sunshine, at the immature eating stage, and at the mature stage contain significant amounts of vitamin A. On the other hand, the white and black varieties contained none or very little of this vitamin.

VITAMIN C

The vitamin C content of the young and full grown plants of the six varieties of maize, used in the vitamin A study, was determined and the data are presented in table 2. From these data it may be seen that the varietal difference in the vitamin C content of maize plants does not vary consistently with the stage of maturity of the plants. There is marked difference in the vitamin C content of the plants as they mature, depending upon whether it is calculated on a moist or a dry basis. On a dry basis all of the varieties exhibited a decrease

in the vitamin C content as the plants matured, but on a moist basis the vitamin C content increased in three of the varieties. There is no correlation between the amount of vitamin A and vitamin C present in maize plants, or between these vitamins and the degree of yellow pigmentation in the kernel.

Table 3 shows that sweet corn at the eating stage is a fairly good source of vitamin C. There is relatively little difference among the varieties examined. The average content of vitamin C is 3.1 I.U. per gram, e.g., 87.7 units per ounce. By comparison fresh orange juice contains approximately 250 units per ounce.

TABLE 2

Effect of maturity of maize plants on vitamin C and moisture content

VARIETY	COLOR	DRY MATTER		VITAMIN C PER GRAM MOIST BASIS		VITAMIN C PER GRAM DRY BASIS	
		Young	Full grown	Young	Full grown	Young	Full grown
		%	%	I.U.	I.U.	I.U.	I.U.
Canada Flint	Yellow	10.6	17.8	10.4	12.6	96.4	81.0
Lancaster Sure Crop	Yellow	9.8	16.9	9.5	18.0	96.8	81.0
Bantam Evergreen	Yellow	10.0	19.0	8.3	7.1	83.4	39.6
Rhode Island Flint	White	10.6	18.0	8.7	10.7	82.4	53.6
Country Gentleman	White	13.7	17.7	14.9	13.2	108.8	80.8
Rustler's White	White	11.0	15.6	....	16.4	....	95.0
Average		10.9	17.5	10.3	13.0	93.5	72.0

EFFECT OF REPORTING RESULTS ON A DRY BASIS

The data presented in tables 1 and 2 support the belief that the vitamin content of plant material should be calculated on a dry rather than on a moist basis. That the variable moisture content of plant material may be responsible for erroneous conclusions relative to the vitamin content is particularly evident from table 2. In this case, the results when calculated on a moist basis show that the vitamin C content of Canada Flint, Rhode Island Flint and Lancaster Sure Crop plants is substantially increased as the plants mature, but in reality there is a marked decrease in the vitamin C content of these varieties, as is shown by the data calculated on a dry basis. These same discrepancies due to variable

moisture content are evident, but not quite so marked, in the changes in the vitamin A content of maize plants, as may be seen in table 1.

As a result of these findings, the authors believe that in comparative vitamin studies, such as are here reported, the moisture content of the samples should be taken into consideration.

TABLE 3

Vitamin A, vitamin C and moisture content of maize kernels at the milk stage and mature stage

VARIETY	COLOR	DRY MATTER		VITAMIN A PER GRAM				VITAMIN C PER GRAM	
				Moist basis		Dry basis			
		Milk stage	Mature	Milk stage	Mature	Milk stage	Mature	Moist basis	Dry basis
		%	%	*I.U.*	*I.U.*	*I.U.*	*I.U.*	*I.U.*	*I.U.*
Golden Bantam	Yellow	36.2	91.1	6.2	6.8	17.3	7.4	3.0	8.4
Bantam Evergreen	Yellow	30.9	91.3	5.6	4.5	17.9	4.9	3.0	9.6
Golden Sunshine	Yellow	37.0	91.0	4.2	2.4	11.3	2.6	3.2	8.6
Country Gentleman	White	18.5	90.9	[1]	[1]	[1]	[1]	[2]	[2]
Stowell's Evergreen	White	21.6	91.9	[1]	[1]	[1]	[1]	[2]	[2]
Black Mexican	Purple	29.5	90.8	[1]	[1]	[1]	[1]	[2]	[2]
Average (for three yellow var.)		34.7	91.1	5.3	4.5	15.5	4.9	3.1	8.8

[1] Less than 1.0 unit per gram. When fed 1.5 gm. daily failed to prevent weight losses.

[2] Vitamin C not determined in these varieties.

VITAMIN D

Harris and Bunker ('34) have found samples of fresh yellow maize from different localities to show marked variations in their rachitogenic properties, which are due apparently to the presence of an antirachitic substance. Templin and Steenbock ('33) noted that immature yellow dent field maize kernels promoted better calcification than corresponding mature maize. Bechtel and Hoppert ('36) investigated the vitamin D contents of dry tassels, silk and leaves of the maize plant at the time of ensiling and found that on a basis of D units per pound of dry matter the tassels, silk and dry leaves contained 1226, 2449 and 2449 units of vitamin D, respectively. Coward

('32) has found that the antirachitic property of green plant material is rapidly lost on storage.

The samples of mature plants which had been frozen for 4 days contained only a very small amount or a trace of vitamin

TABLE 4

Vtiamin D content of maize

VARIETY	COLOR	AMOUNT FED DAILY	NUMBER OF RATS	AVERAGE DEGREE OF CALCIFICATION	VITAMIN D PER GRAM MOIST BASIS
Samples frozen 4 days					
		gm.			*I.U.*
Canada Flint	Yellow	1.0	5	0.6	Trace
Lancaster Sure Crop	Yellow	1.0	5	2.0	2.7–
Bantam Evergreen	Yellow	1.0	5	1.3	Trace
Rhode Island Flint	White	1.0	5	0.1–	Trace–
Country Gentleman	White	1.0	5	0.0	None
Rustler's White	White	1.0	5	0.4	Trace
Samples taken fresh daily					
Canada Flint	Yellow	1.0	8	3.0	2.7
Lancaster Sure Crop	Yellow	1.0	8	2.6	2.7–
Bantam Evergreen	Yellow	1.0	8	2.7	2.7–
Rhode Island Flint	White	1.0	8	2.7	2.7–
Country Gentleman	White	1.0	8	3.0	2.7
Black Mexican	Purple	1.0	6	3.0	2.7
Immature kernels					
Gonden Bantam	Yellow	1.0	6	0.5	Trace
Bantam Evergreen	Yellow	1.0	8	0.0	None
Gonden Sunshine	Yellow	1.0	8	0.1	Trace–
Country Gentleman	White	1.0	8	0.2	Trace
Stowell's Evergreen	White	1.0	8	0.0+	None+
Black Mexican	Purple	1.0	6	0.3	Trace
Positive control	28.5 mg. U.S.P. reference cod liver oil		10	2.9	95
Negative control			5	0.0	None

D whereas the fresh plants contained at least 2.7 I.U. of vitamin D per gram moist or 15 units on a dry basis. This value is equivalent to 6700 units per pound of dry matter. The fresh kernels contained only a trace of vitamin D. These results are in agreement with unpublished data of Coward ('32) who found that the vitamin D content of green plant material was lost rapidly on storage.

CONCLUSIONS

1. There is a definite increase in the vitamin A content of maize plants as they grow. Average values for 25 cm. plants and plants after they had attained maximum growth were 49 and 76 units per gram, respectively (dry basis).

2. The vitamin C content of these same maize plants decreased from 93.5 to 72 units per gram (dry basis).

3. There is no correlation between the vitamin A and C content of the plant and the color of the kernels of maize plants.

4. Only the yellow pigmented kernels of maize plants contain demonstrable amounts of vitamin A.

5. Fresh maize plants contain an antirachitic substance which is lost rapidly on storage. The fresh immature or ripe grain kernels contain only a trace of vitamin D.

6. The importance of reporting vitamin data in plant material in terms of dry matter, in order to avoid possible erroneous conclusions, is stressed.

7. There is a marked loss in vitamin A and moisture in maize plants after they have attained maximum growth. This loss of vitamin A is a factor which should be taken into consideration when maize is grown as a feed for livestock.

8. Kernels of yellow maize lose much of their vitamin A as they mature and dry out.

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THE EFFECT OF THE ORAL ADMINISTRATION OF PANCREATIN ON FECAL NITROGEN AND FAT LOSS IN ACHYLIA PANCREATICA

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In a previous publication we have shown that the oral administration of diastase decreases the starch lost in the feces in achylia pancreatica (Beazell, Schmidt and Ivy, '37). A review of the literature reveals that complete exclusion of pancreatic juice from the intestine increases the loss of fat and nitrogen in the feces (Pratt, Lamson and Marks, '09; Cruickshank, '15; Maltby, '31; Nasset, Pierce and Murlin, '31; Handelsman, Golden and Pratt, '34; Ivy, '35; and Selle, '37) and that substitution therapy has been uniformly effective in reducing the loss of nitrogen (Pratt, Lamson and Marks, '09; Cruickshank, '15; Nasset, Pierce and Murlin, '31; and Selle, '37). The value of orally administered enzymes in reducing the loss of fat remains, however, unsettled (Selle, '37). In this study we have investigated the effect of oral enzyme therapy on the loss of nitrogen and fat in the feces in pancreatic achylia.

METHODS

In seven dogs weighing from 11 to 14 kilos the pancreatic juice was totally excluded from the intestine by separating the pancreas from all of its connections with the duodenum. Post-operatively the dogs were fed a nutritious diet containing 200 gm. of raw pancreas for a period of a month or longer. This diet maintained them in excellent condition.

Submaintenance diet. Five of the animals were then given a submaintenance diet consisting of 200 gm. of raw, lean meat daily for a period of 21 days. The feces were discarded for the first 2 days after which collections were made for the next 5 days. This constituted the first control period. Twenty-five grams of pancreatin, in the form of enteric coated tablets, were then added to the daily diet of each dog. Forty tablets were given with the meal, twenty more at 2 hours, ten at 4 hours, and five at 6 hours post-cibum. The feces were again discarded for the first 2 days and collected for the following 5 days. This constituted the 'pancreatin' period. The pancreatin was then discontinued, and after discarding the feces for 2 days, collections were again made for 5 days. This constituted the second control period. The total and dry weights and the nitrogen content of the feces was determined daily. Nitrogen determinations were made in duplicate on the dried feces by the Kjeldahl method.

Maintenance diet. The animals were then returned to the diet containing raw pancreas for several months. Dogs nos. 3 and 4 used above and two dogs not previously used were then given a standard diet of 600 gm. of cooked ground beef heart from which all visible fat had been removed. The diet was prepared in 50 pound batches and was preserved in the ice box. Nitrogen determinations were made in duplicate on aliquots from each batch.

As before the experiment was divided into three 5-day metabolic periods. During the first and last periods the feces were collected while the animals were on the diet (control periods). During the second period 25 gm. of pancreatin, in the form of enteric coated tablets, were given in a single dose with the daily diet. A carmine 'marker' was given mixed with the meal on the third day of each period and again on the eighth day. The feces were collected daily starting with the first appearance in the stool of the first dose of carmine and ending with the first appearance of the second dose.

The 24-hour excretion of feces from each dog was weighed daily and, after thorough mixing, aliquots were taken for the

fat and nitrogen determinations. The samples for each dog were pooled during each metabolic period and determinations were made on the composite sample. The samples for the nitrogen determination were suspended in sulphuric acid as recommended by Peters and Van Slyke ('32); samples for the fat determination were preserved in alcohol. Nitrogen was determined by the Kjeldahl method, fat by a modification of Saxon's method (Peters and Van Slyke, '32). All determinations were made in duplicate. No correction was made for the bulk or metabolite content of the pancreatin.

The tryptic activity of the pancreatin used throughout this study was from 50% to 100% greater than that required by the U.S.P. The lipase activity was determined by the method described by Cherry and Crandall ('32). The activity, expressed as the number of cubic centimeters of 0.2 N. NaOH required to neutralize the fatty acids liberated by 1 gm. of pancreatin acting at optimum concentration, was 2200 units.

RESULTS

Control periods. The feces passed during the control periods were, in most instances, bulky and characteristic of pancreatic achylia. The individual nitrogen excretion values checked remarkably well for the first and third control metabolic periods. On the maintenance diet the average nitrogen utilization was 69.2% for the first period, and 71.5% for the third period (table 2). Fecal fat values showed greater individual variations, although averages for the two periods compare favorably. During the first 5-day control period an average of 50.0 gm. of fat (14.6 to 78.7 gm.) was eliminated; during the third period, 47.0 gm. (9.9 to 86.1 gm.) (table 3).

Pancreatin period. The addition to the diet of 25 gm. of enteric coated pancreatin tablets had an inconstant effect on the dry weight of the feces. In the experiment in which dry weight was determined, three of the dogs showed an increase while the other two showed a decrease (table 1). The effect of pancreatin on the wet weight of the feces was more constant. On the submaintenance diet the wet weight of the feces was

TABLE 1

Effect of pancreatin on fecal nitrogen excretion. Five dogs with pancreatic achylia on a submaintenance diet (values are totals for entire metabolic period)

DOG NO.	FIRST METABOLIC PERIOD 5 DAYS—CONTROL				SECOND METABOLIC PERIOD 5 DAYS—25 GM. PANCREATIN DAILY							THIRD METABOLIC PERIOD 5 DAYS—CONTROL			
	Wet weight feces	Dry weight feces	Per cent solids	Total fecal nitrogen	Wet weight feces	Dry weight feces	Per cent solids	Total fecal nitrogen	Per cent change solids[1]	Per cent change bulk[1, 2]	Per cent decrease nitrogen[1]	Wet weight feces	Dry weight feces	Per cent solids	Total fecal nitrogen
1	1013	261	25.7	18.1	691	333	48.2	8.6	+18.0	—41.0	60.0	1330	304	22.9	25.2
2	1463	438	29.9	32.3	474	199	42.1	12.0	—53.8	—63.3	62.5	1120	425	37.9	31.7
3	480	137	28.5	13.4	545	234	42.9	6.1	+61.5	+4.2	57.2	567	153	27.0	14.8
4	135	49.5	36.6	5.0	341	131	38.4	4.1	+150.0	+154.0	21.0	133	55	41.3	5.5
5	1006	254	25.2	22.3	502	197	39.3	6.7	—12.4	—46.7	65.8	874	197	22.6	17.2
Average	819	228	27.9	18.2	510	219	42.9	7.5	—3.74	—37.2	59.5	805	227	28.2	18.8

[1] Based on average of two control periods.

[2] Based on wet weight of feces.

TABLE 2

Effect of pancreatin on fecal nitrogen and bulk. Four dogs with pancreatic achylia on a maintenance diet (values are totals for entire metabolic period)

DOG NO.	FIRST METABOLIC PERIOD 5 DAYS—CONTROL				SECOND METABOLIC PERIOD 5 DAYS—25 GM. PANCREATIN DAILY						THIRD METABOLIC PERIOD 5 DAYS—CONTROL			
	Wet weight feces	Nitrogen ingested	Fecal nitrogen	Nitrogen utilized	Wet weight feces	Nitrogen ingested	Fecal nitrogen	Nitrogen utilized	Change in bulk[1]	Reduction in N loss[1]	Wet weight feces	Nitrogen ingested	Fecal nitrogen	Nitrogen utilized
		gm.	*gm.*	%		*gm.*	*gm.*	%	%	%		*gm.*	*gm.*	%
3	597	110	22.1	79.5	421	107.7	13.2	87.7	—26.2	35.3	545	113	18.7	83.5
4	280	110	13.8	87.4	340	107.7	8.7	91.7	+12.9	31.5	322	113	11.6	89.7
6	1184	110	49.3	55.4	479	107.7	13.4	87.6	—59.5	73.4	1181	113	49.9	56.0
7	1142	110	50.6	54.1	584	107.7	18.9	82.5	—46.5	61.8	1046	113	48.4	57.3
Average	801	110	33.9	69.2	456	107.7	13.5	87.5	—42.2	59.2	773	113	32.2	71.5

[1] Based on average of two control periods.

decreased in only three of the five dogs studied, but the average change indicates a substantial decrease (37.2%). On the maintenance diet three dogs showed a decrease and one an increase, the average being a 42.2% decrease. Fecal nitrogen and fat were decreased significantly in all of the animals studied. Nitrogen loss was reduced 21 to 66% (average 59.5%) on the submaintenance diet and 31 to 73% (average 59.2%) on the maintenance diet (tables 1 and 2). Fecal fat loss was reduced 14 to 78% (average 59.1%) (table 3).

It will be seen by comparing the values in tables 1 and 2 that orally administered pancreatin exerts its greatest effect in those animals showing the greatest nitrogen and fat loss

TABLE 3

Effect of pancreatin on fecal fat excretion. Maintenance diet (600 gm. lean beef heart daily)

DOG NO.	TOTAL LIPOID IN FECES (GM.)			PERCENTAGE REDUCTION IN FAT LOSS EFFECTED BY PANCREATIN (TWO CONTROL PERIODS AVERAGED)
	First metabolic period (control)	Second metabolic period (pancreatin)	Third metabolic period (control)	
3	33.4	15.6	19.4	40.8
4	14.6	10.4	9.9	14.7
6	78.7	17.9	86.1	78.3
7	73.0	35.3	72.6	51.5
Average	50.0	19.5	47.0	59.1

during the control periods (dogs 1, 2, 5, 6, 7), and the least effect in those animals which tolerate pancreatic achylia well (dogs 3, 4). Dogs 3 and 4 have been maintained for a year and a half with pancreatic achylia and may represent an adaptation that occurs under such conditions. This seems likely in the case of dog 3 which on a submaintenance diet showed a fairly high nitrogen excretion that was decreased 57.2% by pancreatin (table 1). Nine months later, on a maintenance diet this animal showed a relatively low nitrogen excretion which was reduced only 35.3% when pancreatin was added to the diet. In any event, the nitrogen and fat utilization in all of the animals reached approximately the same level when adequate substitution therapy was instituted.

DISCUSSION

In depancreatized dogs, according to Cruickshank ('15), the administration of raw pancreas (150 gm.) increased nitrogen utilization from a control value of 78% to 92%. Our findings on the effect of pancreatin on nitrogen loss in dogs deprived of the external pancreatic secretion confirm previous reports. Nasset, Pierce and Murlin ('31) reported that pancreatin increased nitrogen utilization in two depancreatized dogs from a control value of 62% to 72% in one, and from 78% to 85% in the other. Selle ('37) reported that pancreatin decreased nitrogen loss from 30 to 60% in ten diabetic dogs maintained on insulin. In our studies of four dogs on a maintenance diet the average nitrogen utilization was 70.5% without pancreatin and 87.5% with pancreatin. The average reduction in nitrogen loss was 59.5% in five dogs on a submaintenance diet and 59.2% in four dogs on a maintenance diet.

Our findings on the effect of pancreatin on fat excretion in pancreatic achylia are in contrast to those of Selle who reported that pancreatin had no effect on fat excretion in diabetic dogs. In our dogs, the average reduction in fecal fat loss was 59.1%, variations from animal to animal being quite marked. Since an appreciable amount of fecal fat represents endogenous fat which is essentially unrelated to the fat of the diet, and since fat mobilization in diabetic animals, even when controlled with insulin, is abnormal, it is not unlikely that pancreatin would effect fecal fat differently in diabetic and nondiabetic dogs. The fed fat would have to be 'labeled' with heavy hydrogen to answer this question.

On the basis of these findings, together with those on starch digestion reported by us in a previous paper (Beazell, Schmidt and Ivy, '37), it would appear that pancreatin decreases to a comparable degree the loss of protein, fats, and starches in pancreatic achylia (50 to 60%). The decrease in moisture content of the feces by pancreatin may be due to either an osmotic effect or a chemical irritative effect exerted by the undigested or partially digested foods.

SUMMARY AND CONCLUSIONS

The pancreatic juice was completely excluded from the intestine in seven dogs. For a period of a month or more the animals were maintained in excellent condition on a mixed diet and raw pancreas. Five dogs were then placed on a submaintenance diet of 200 gm. lean beef for three consecutive 7-day periods (21 days). Pancreatin (25 gm.; potency two times U.S.P.) was given during the second 7-day period. This procedure was repeated with four dogs on a maintenance diet of 600 gm. of trimmed beef heart. Analysis of the feces for nitrogen and fat showed that pancreatin decreased the elimination of these constituents about 60% and 59%, respectively, regardless of the quantity of diet fed. Fecal bulk was reduced 37 to 42%, and the stools became better formed and drier. It is concluded that potent pancreatin, when given in the form of enteric coated tablets and in adequate amount is significantly effective in reducing nitrogen and fat loss in the feces of dogs having achylia pancreatica.

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THE UTILIZATION OF ENERGY PRODUCING NUTRIMENT AND PROTEIN AS AFFECTED BY DEFICIENCY OF IRON AND COPPER [1]

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The deficiency of milk in iron and copper having been first demonstrated by Hart, Steenbock, Waddell and Elvehjem ('28) to be the cause of the whole milk anemia of the albino rat, the study to be discussed was an investigation of these associated nutritive deficiencies, and the resulting anemia, as they affect the efficiency of utilization of food energy and protein.

In this relation the concern of the authors with the development of symptoms of anemia was only as this condition bore definite evidence that the iron and copper deficiency in the food, the effects of which were to be studied, had become nutritionally effective.

In the production of milk anemia in young rats Elvehjem and Kemmerer ('31) have recommended that they be prevented from consuming any food other than milk, during the suckling period; and Levine, Culp and Anderson ('32) placed the rats on a milk diet as soon as the average body weight of the animal reached 40 gm. (approximately 21 days of age), in order to diminish body stores of iron and copper as rapidly as possible.

A spray process, whole milk powder (Klim) has been found more convenient than liquid whole milk, for this purpose, by

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Harris ('32), Levine, Culp and Anderson ('32), and Remington ('33), since a sufficient quantity of the product, of uniform composition, can be obtained to last for an entire experiment. These workers report satisfactory results from feeding the milk powder in a dry condition; and this method of feeding milk is especially desirable when an accurate record of the food consumption is wanted. The group of workers above mentioned report the iron content of the milk powder as varying between 2.4 and 5 parts per million, and the copper content between less than 1 and 2 parts per million.

Harris ('32) observed that rats fed a ration of whole milk powder (Klim) developed diarrhea, which usually cleared up by the seventh day after weaning. He attributed this condition to the high lactose content of milk.

Palmear and Kennedy ('31) found the digestibility of whole milk powder to be slightly higher than that of liquid milk, the values being 95.6% and 92.7%, respectively.

The prolonged feeding of a milk-iron-copper diet to rats was found by Underhill, Orten, Mugrage and Lewis ('33) to be without harmful effects, though the body weight attained was somewhat less than normal.

Among the observations which have been made on the growth of rats with nutritional anemia, Elvehjem and Kemmerer ('31) were able to produce severe anemia within 2 weeks after weaning, when special precautions were observed not to allow the young rats to receive any food other than liquid milk. The body weight of these animals increased very little after they were weaned; and death usually resulted by the time they had been weaned 4 weeks.

Supplee, Dow, Flanigan and Kahlenberg ('30) found that rats which received reconstituted, spray process, dry milk, and had initial weights of approximately 45 gm., and blood hemoglobin of 14 gm., increased in weight to 110 gm. during 10 weeks, the hemoglobin value decreasing to about 4.5 gm. during this time.

Harris ('32) fed a group of 100 weanling albino rats for a period of 9 weeks on a whole milk powder (Klim) ration. The

approximate initial and final live weights of the rats (as indicated in a graph) were 35 and 170 gm., and the blood hemoglobin values 11.0 and 2.8 gm., respectively.

Remington ('33) states that the inverse relationship between rate of growth and blood hemoglobin value, as reported by Harris, is in agreement with the findings of others. Andes and Beard ('34) found that ten rats, which ingested an average of 38 cc. of milk per day, increased in weight from 92.0 gm. to 160.5 gm. in 10 weeks. During the same period the hemoglobin content of the blood decreased from 3.9 gm. to 3.6 gm. per 100 cc.

It has been reported by Krauss ('31) that rats fed exclusively on whole milk had enlarged hearts, lungs, spleens and kidneys, but smaller testes than normal. The observation of hypertrophy of cardiac muscle has been confirmed by Daniels and Burright ('33), who found this condition continuing after the rats had been cured of the anemia.

The experiment to be reported was devised as a unit in a series of investigations of the efficiency of food utilization as affected by specific nutrient deficiencies—in this case, of iron and copper; this phase of nutritional anemia not having been investigated, heretofore, by carefully controlled experimentation.

The method of procedure followed was the same as in previous studies of this series, and as described by Swift, Kahlenberg, Voris and Forbes ('34).

PRELIMINARY EXPERIMENT

In the first start of this experiment a diet of whole milk powder (spray process), dextrin, casein, Crisco and vitamin B concentrate was fed. It was found, however, that the dextrin and Crisco contained considerable amounts of iron; and on this account the rats did not develop nutritional anemia. This false start, therefore, was abandoned, and the experiment was begun again on the better basis to be described.

EXPERIMENTAL PROCEDURE

Two rations of whole milk powder (Klim), one unsupplemented, and the other supplemented with iron and copper, were compared in a growth, metabolism and body balance study with twelve pairs of weanling albino rats. The pair mates were of the same sex, from the same litter, and of approximately the same body weight at the start of the experiment.

In order to develop the nutritional anemia as rapidly as possible the mothers of the experimental subjects were fed milk powder alone from the time the young rats were 7 days of age until they were weaned at the age of 21 days. Also, the experimental cages and feeders were lacquered, and the animals were not allowed access to any copper or iron; and, further, all water given the rats while on experiment was redistilled in glass.

TABLE 1

Gain in body weight[1] *of rats as related to dry matter of food*

PAIR	SEX	Fe + Cu DEFICIENT DIET			Fe + Cu SUPPLEMENTED DIET		
		Food eaten (dry matter)	Gain in body weight	Dry matter of food per gram body gain	Food eaten (dry matter)	Gain in body weight	Dry matter of food per gram body gain
		gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
1	♂	187.4	37.65	5.0	187.1	45.21	4.1
2	♂	192.4	46.99	4.1	192.1	56.70	3.4
3	♀	202.4	50.61	4.0	202.1	52.95	3.8
4	♂	187.0	43.37	4.3	186.7	56.93	3.3
5	♂	174.2	42.99	4.1	173.9	53.52	3.2
6	♂	184.1	52.88	3.5	183.8	58.98	3.1
7	♀	182.8	35.43	5.2	182.5	42.62	4.3
8	♀	200.3	41.62	4.8	200.0	43.11	4.6
9	♂	183.0	36.85	5.0	182.7	44.49	4.1
10	♀	183.5	43.46	4.2	183.2	45.15	4.1
11	♀	202.5	51.53	3.9	202.2	50.82	4.0
12	♀	200.5	46.85	4.3	200.2	52.44	3.8
Average		190.0	44.19	4.3	189.7	50.24	3.8

[1] Excluding contents of alimentary tract.

Since most of the rats had diarrhea when weaned, they were not started on experiment until 26 days of age (average)

in order to allow time for this disorder to clear up. Some trouble on this account continued, with a few of the rats, however, the diarrhea persisting, in a mild form, for from 4 to 5 weeks.

TABLE 2

Energy of body gain of rats as related to energy, and to metabolizable energy of food

PAIR	FEED ENERGY	Fe + Cu DEFICIENT DIET				Fe + Cu SUPPLEMENTED DIET			
		Body gain		Metabolizable energy[1]	Body gain as percentage of metabolizable energy	Body gain		Metabolizable energy[1]	Body gain as percentage of metabolizable energy
			Per cent of feed energy				Per cent of feed energy		
	Cal.	*Cal.*		*Cal.*	%	*Cal.*		*Cal.*	%
1	1054.2	56.8	5.4	930.9	6.1	75.0	7.1	917.4	8.2
2	1082.5	87.8	8.1	953.0	9.2	139.4	12.9	961.8	14.5
3	1138.9	111.7	9.8	1020.3	10.9	140.2	12.3	1011.9	13.9
4	1052.0	72.6	6.9	928.3	7.8	137.5	13.1	921.4	14.9
5	980.1	87.9	9.0	877.1	10.0	131.8	13.4	871.4	15.1
6	1036.0	106.2	10.3	938.2	11.3	146.9	14.2	915.1	16.1
7	1028.2	87.2	8.5	914.8	9.5	101.7	9.9	881.0	11.5
8	1126.7	78.7	7.0	983.5	8.0	116.3	10.3	954.1	12.2
9	1029.3	50.8	4.9	945.4	5.4	97.3	9.5	899.4	10.8
10	1032.1	112.2	10.9	930.6	12.1	103.9	10.1	902.4	11.5
11	1139.5	94.8	8.3	1040.6	9.1	153.2	13.4	977.2	15.7
12	1127.8	102.7	9.1	984.3	10.4	168.2	14.9	1007.9	16.7
Ave.	1068.9	87.5	8.2	953.9	9.2	126.0	11.8	935.1	13.5

[1] Uncorrected for non-metabolizable energy of protein gained.

The same lot of whole milk powder (Klim) was used in both rations during the entire experiment. The milk powder was of a special lot which had been recommended by Remington (personal communication) as being consistently low in iron and copper.

The supplemented ration contained in each 5 gm. 0.5 mg. of iron in the form of $Fe\ Cl_3\ .6H_2O$, and 0.05 mg. of copper in the form of $CuSO_4.5H_2O$.

The average food consumption was somewhat less than 5 gm. per day, the smallest average being 4.2 gm. However, this amount of feed provided enough of the iron and copper supplements to cure nutritional anemia.

The food intake was controlled by the paired method, and the two rations contained equal amounts of nitrogen and energy per gram.

The experiment was brought to a close, on account of marked decrease in food consumption, after 38 days' feeding of pair no. 9, and 42 days' feeding of the remaining eleven pairs.

At the end of the experiment the rats were killed, and chemical analyses were made of the rat bodies, and of the food, urine and feces.

TABLE 3

Heat loss related to energy of food

PAIR	FEED ENERGY	Fe + Cu DEFICIENT DIET		Fe + Cu SUPPLEMENTED DIET	
		Heat loss		Heat loss	
			Per cent of feed energy		Per cent of feed energy
	Cal.	*Cal.*		*Cal.*	
1	1054.2	874.1	82.9	842.4	79.9
2	1082.5	865.2	79.9	822.4	76.0
3	1138.9	908.6	79.8	871.7	76.5
4	1052.0	855.7	81.3	783.9	74.5
5	980.1	789.2	80.5	739.6	75.5
6	1036.0	832.0	80.3	768.2	74.2
7	1028.2	827.6	80.5	779.3	75.8
8	1126.7	904.8	80.3	837.8	74.4
9	1029.3	894.6	86.9	802.1	77.9
10	1032.1	818.4	79.3	798.5	77.4
11	1139.5	945.8	83.0	824.0	72.3
12	1127.8	881.6	78.2	839.7	74.5
Average	1068.9	866.5	81.1	809.1	75.7

The body gains of nitrogen and energy and the heat production were computed by a difference procedure. To obtain the composition of the rat bodies at the start of the experiment, a group of control animals, of similar weight and treatment, was killed and analyzed at this time. The gains of nitrogen and energy were computed as the difference between the final analyses of the experimental animals and the estimated amount of nitrogen and energy which these animals contained at the start of the experiment. The heat production was computed by subtracting from the gross energy of the feed the sum of the energy equivalents of the feces, urine and body gain.

The experimental procedure was as described, in detail, by Swift, Kahlenberg, Voris and Forbes ('34), and by Forbes, Swift, Black and Kahlenberg ('35).

TABLE 4

Nitrogen of body gain related to fat and energy of body gain, and to nitrogen of feed

PAIR NO.	NITROGEN OF BODY GAIN	Fat gained		Energy gained			Nitrogen of feed	
		Total	Per gram nitrogen gained	Total	As protein	As fat		Utilized for body gain
	gm.	*gm.*	*gm.*	*Cal.*	*Cal.*	*Cal.*	*gm.*	%
		Fe + Cu DEFICIENT DIET						
1	1.54	0.6	0.4	56.8	51.3	5.5	7.37	20.9
2	1.64	3.6	2.2	87.8	53.8	34.0	7.57	21.7
3	1.77	5.7	3.2	111.7	58.3	53.4	7.96	22.2
4	1.45	2.7	1.9	72.6	47.7	24.9	7.36	19.7
5	1.43	4.3	3.0	87.9	47.2	40.7	6.85	20.9
6	1.73	5.2	3.0	106.2	57.4	48.8	7.24	23.9
7	1.09	5.6	5.1	87.2	35.4	51.8	7.19	15.2
8	1.49	3.4	2.3	78.7	46.8	31.9	7.88	18.9
9	1.38	0.6	0.4	50.8	44.9	5.9	7.20	19.2
10	1.48	6.6	4.5	112.2	50.5	61.7	7.22	20.5
11	1.78	3.9	2.2	94.8	59.2	35.6	7.97	22.3
12	1.41	5.8	4.1	102.7	48.4	54.3	7.89	17.9
Average	1.52	4.0	2.6	87.5	50.1	37.4	7.48	20.3
		Fe + Cu SUPPLEMENTED DIET						
1	1.89	1.3	0.7	75.0	62.4	12.6	7.37	25.6
2	2.03	7.7	3.8	139.4	67.0	72.4	7.57	26.8
3	1.77	8.5	4.8	140.2	60.9	79.3	7.96	22.2
4	1.99	7.4	3.7	137.5	68.8	68.7	7.36	27.0
5	1.92	7.2	3.8	131.8	63.6	68.2	6.85	28.0
6	2.04	8.0	3.9	146.9	71.5	75.4	7.24	28.2
7	1.54	5.2	3.4	101.7	53.0	48.7	7.19	21.4
8	1.50	6.6	4.4	116.3	54.2	62.1	7.88	19.0
9	1.80	4.0	2.2	97.3	59.3	38.0	7.20	25.0
10	1.70	5.2	3.1	103.9	55.0	48.9	7.22	23.5
11	1.74	10.2	5.9	153.2	58.5	94.7	7.97	21.8
12	1.72	11.8	6.9	168.2	58.2	110.0	7.89	21.8
Average	1.80	6.9	3.8	126.0	61.0	64.9	7.48	24.1

Iron was determined in the milk powder by the method of Elvehjem, as described by Farrar ('35), and copper by the potassium ethyl xanthate method which was adopted for determination of copper in milk by Supplee and Bellis ('22).

The hemoglobin content of the blood was determined at the end of the second, third and sixth weeks of the experiment, by the colorimetric acid hematin method of Newcomer, with a glass standard.

TABLE 5

Digestibility of nitrogen and energy producing nutriment

PAIR	Fe + Cu DEFICIENT DIET				Fe + Cu SUPPLEMENTED DIET			
	Nitrogen		Energy		Nitrogen		Energy	
	Feces	Digested	Feces	Digested	Feces	Digested	Feces	Digested
	gm.	%	*Cal.*	%	*gm.*	%	*Cal.*	%
1	0.63	91.5	75.3	92.9	0.92	87.5	97.0	90.8
2	0.66	91.3	84.7	92.2	0.75	90.1	81.0	92.5
3	0.54	93.2	72.2	93.7	0.75	90.6	82.1	92.8
4	0.67	90.9	75.1	92.9	0.82	88.9	85.9	91.8
5	0.67	90.2	63.5	93.5	0.72	89.5	73.2	92.5
6	0.60	91.7	56.0	94.6	0.80	89.0	84.5	91.8
7	0.60	91.7	57.0	94.5	0.89	87.6	93.0	91.0
8	0.72	90.9	84.6	92.5	1.00	87.3	118.4	89.5
9	0.46	93.6	37.7	96.3	0.86	88.1	92.1	91.1
10	0.71	90.2	52.2	94.9	0.97	86.6	87.7	91.5
11	0.64	92.0	50.5	95.6	1.10	86.2	114.4	90.0
12	0.60	92.4	61.7	94.5	0.83	89.5	73.8	93.5
Average	0.63	91.6	64.2	94.0	0.87	88.4	90.3	91.6

The energy content of both diets was determined, by analysis, to be 5534 Calories per gram, and the nitrogen content, 3.87%. The moisture content of the deficient ration was 1.63%, and of the supplemented ration, 1.79%. The iron and copper contents of the whole milk powder were 3 parts, and 1½ parts per million, respectively.

PRESENTATION OF RESULTS

There was a total of 242 refusals of feed during the course of the experiment, as food was given each pair of rats until one individual refused. Of these refusals 225 were by the rats which received the deficient diet, and only seventeen by the rats on the supplemented diet, a difference that would occur by chance once only in billions of trials. Thus the anemic rats very definitely restricted the food intake of their pair mates.

The average gains in body weight, excluding the contents of the alimentary tract, were 44.19 gm. for the deficient rats, and 50.24 gm. for the supplemented rats. Statistical treatment reveals odds of 5000 to 1 that the gain made by the supplemented rats was significantly greater than that made by the deficient rats. Since the energy intake of each pair of rats was identical, there is a correspondingly more efficient utilization of food by the supplemented rats, which is represented in the dry matter of food per gram of body gain.

The energy storage by the supplemented rats was greater than by the deficient rats in eleven pairs among the twelve. The average energy gains were 126.0 Calories for the supplemented rats, and 87.5 Calories for the deficient rats; and the odds that this difference is significant are over 10,000 to 1. The difference between the metabolizable energy values of the diets for the two groups of rats was very small, but the odds that this difference was significant were 100 to 1. Consequently there was a higher percentage of metabolizable energy utilized for body gain by the supplemented than by the deficient rats.

The values reported for metabolizable energy in this experiment were not corrected for the non-metabolizable fraction of the energy stored as protein. The derivation of these values is simply the gross energy of the feed minus the energy of the feces and urine.

The iron-and-copper deficient rats, in a state of nutritional anemia, produced more heat in every case than did their pair mates, the blood of which was maintained normal by the addition of iron and copper to the diet. The significance of this difference is expressed by odds of over 10,000 to 1.

The group of rats which gained the most energy (the supplemented rats) must necessarily have produced less heat than the other, since both groups of animals metabolized essentially the same quantities of food energy.

It is possible that the increased heat production of the deficient animals resulted either from increased activity, or from increased energy expense of supplying the tissues with oxygen.

Of the energy retained by the deficient rats, 50.1 Calories were gained as protein, and 37.4 Calories as fat; while with the supplemented rats, 61.0 Calories were gained as protein, and 64.9 Calories as fat. The supplemented rats gained larger absolute quantities of protein (odds 5000 to 1), but with the deficient rats a higher percentage of the gain of energy was in the form of protein.

The supplemented rats gained an average 6.9 gm. of fat, while the deficient rats gained only 4.0 gm. The significance of this difference is expressed by odds of approximately 1600 to 1.

The addition of the iron and copper compounds to the milk powder unfavorably affected its digestibility. The average digestibility of nitrogen was 91.6% and 88.4%, and of the energy 94.0% and 91.6%, with the deficient and the supplemented rats, respectively. The odds representing the significance of these differences are 3000 to 1 for the digestibility of energy, and over 10,000 to 1 for the digestibility of nitrogen. The urine of the deficient rats, therefore, should contain more energy than should that of the supplemented rats, since the metabolizable energy was essentially the same for both groups, and the digestibility was the higher for the deficient group. This suggestion was found to be correct—the urine of the deficient rats containing an average of 50.8 Calories, while that of the supplemented rats contained 43.6 Calories.

The moisture content of the rat bodies was higher for the deficient rats than for the supplemented rats in eleven pairs among the twelve; the average being 66.0 $\pm$ 0.28% and 63.0 $\pm$ 0.34%, respectively. The significance of this difference is expressed by odds of approximately 4666 to 1. The greater gain of energy by the supplemented rats, therefore, resulted from a greater gain in live weight, containing a higher percentage of dry matter.

From the time the first blood hemoglobin values were determined, 2 weeks after the experiment started, the deficient animals, which received milk powder alone, manifested symptoms of severe nutritional anemia; while the rats which

received the supplements of iron and copper exhibited normal blood hemoglobin values.

The hemoglobin estimations on the blood of the anemic animals were not wholly satisfactory, since the standard colorimeter available at the time a part of these determinations were made was not well adapted to the purpose—the plunger barely touching the liquid, when comparisons were being made with the Newcomer plate.

A micro-colorimeter, designed especially for this type of work, was used for a part of the hemoglobin determinations made at the end of the experiment. Readings were made with both types of colorimeters on the same solutions, and the earlier readings were corrected, by computation, to the basis of the more satisfactory micro-colorimeter determinations. These corrected blood hemoglobin values, therefore, are reported not for a critical study, but only as indicative of the general condition of the rats.

Grams Hb per 100 cc. of blood (average values)

	Fe + Cu deficient rats	*Fe + Cu supplemented rats*
Second week	2.97 ± 0.07	14.28 ± 0.11
Third week	2.93 ± 0.09	14.86 ± 0.19
Sixth week	2.80 ± 0.04	16.10 ± 0.20

The number of blood samples taken from each rat was limited to a minimum, as the taking of the sample occasioned some disturbance to the animal. In such experiments it is desirable to avoid such disturbances, as largely as possible.

When the animals were killed at the end of the experiment, the following observations were made. The skin of the anemic animals was colorless, the hair chalk-white, and the eyes extremely pale. The livers, lungs, hearts and kidneys were also markedly deficient in color. The hearts of all the deficient animals were greatly enlarged; and in ten cases among the twelve, the spleens and kidneys were noticeably larger in the deficient animals. The spleen in the anemic rats was usually enlarged to two or three times the size of the spleen of the normal animals; and the caecum of one of the deficient rats contained evidence of intestinal hemorrhage.

CONCLUSION

In a 6-week growth, metabolism, and body balance experiment, with twenty-four young, growing, albino rats as subjects, an iron-and-copper-deficient diet (whole milk powder) was compared with another diet differing only in that it was supplemented with iron and copper salts. Feeding was controlled by the paired method.

The rats which received the iron-and-copper-deficient diet promptly developed typical cases of nutritional anemia. These rats digested more of the energy and nitrogen of the ration, produced more heat, and stored more water than did those which received the iron and copper supplements, and also limited the food consumption of their normal pair mates.

The rats which received the supplements of iron and copper, made greater gains of body weight, stored more energy, and gained more fat and nitrogen than did the anemic rats, which consumed the same quantities of nitrogen and energy.

The difference between the metabolizable energy values of the diets for the two groups of rats was very small, but the odds that this difference was significant were 100 to 1.

The hearts, spleens and kidneys of the iron-and-copper-deficient animals were generally much enlarged.

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